

P:35



(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication and mention
of the grant of the patent:
02.04.2003 Bulletin 2003/14

(51) Int Cl.7: **C12N 15/13, C12N 15/63**
// C07K16/30

(21) Application number: **97200482.4**

(22) Date of filing: **24.09.1992**

(54) **Anti-human milk fat globule humanised antibodies and processes for their production**

Anti-menschliche MilCHFettglobule humanisierte Antikörper und Verfahren zu ihrer Herstellung

Anticorps humanisés anti-globule de matière grasse du lait et procédé de préparation

(84) Designated Contracting States:
AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL
PT SE

(30) Priority: **26.09.1991 GB 9120467**

(43) Date of publication of application:
02.07.1997 Bulletin 1997/27

(62) Document number(s) of the earlier application(s) in
accordance with Art. 76 EPC:
92308680.5 / 0 534 742

(73) Proprietor: **CELLTECH THERAPEUTICS LIMITED**
Slough, Berkshire SL1 3WE (GB)

(72) Inventors:
• **Hamann, Philip R.**
Garnerville, New York 10923 (US)
• **Adair, John Robert**
High Wycombe, Bucks., HP14 3RN (GB)

- **Owens, Raymond John**
Henley-On-Thames, Oxon., RG9 1SH (GB)
- **Baker, Terence Seward**
Wraysbury, Staines, Middlesex, TW19 5JF (GB)
- **Lyons, Alan Howard**
Maldenhead, Berks. SL6 3XN (GB)
- **Hinman, Lois M.**
North Tarrytown, New York 10591 (US)
- **Menendez, Ana T.**
Monmouth Junction New Jersey 08852 (US)

(74) Representative: **Mercer, Christopher Paul**
Carpmaels & Ransford
43, Bloomsbury Square
London WC1A 2RA (GB)

(56) References cited:
EP-A- 0 208 615 **EP-A- 0 392 384**
WO-A-91/09967

EP 0 781 845 B1

Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

Description**FIELD OF THE INVENTION**

- 5 **[0001]** The present invention relates to humanised antibody molecules (HAMs) having specificity for human milk fat globule (HMFG) and to processes for their production using recombinant DNA technology.

BACKGROUND TO THE INVENTION

- 10 **[0002]** The term "humanised antibody molecule" (HAM) is used to describe a molecule having an antigen binding site derived from an immunoglobulin from a non-human species, the remaining immunoglobulin-derived parts of the molecule being derived from a human immunoglobulin. The antigen binding site may comprise: either a complete variable domain from the non-human immunoglobulin fused onto one or more human constant domains; or one or more of the complementarity determining regions (CDRs) grafted onto appropriate human framework regions in the variable domain. The abbreviation "MAb" is used to indicate a monoclonal antibody.

15 **[0003]** In the description, reference is made to publications by number. These numbers are placed in square brackets []. The publications are listed in numerical order at the end of the description.

- 20 **[0004]** Natural immunoglobulins have been known for many years, as have the various fragments thereof, such as the Fab, Fab' (Fab')₂ and Fc fragments, which can be derived by enzymatic cleavage. Natural immunoglobulins comprise a generally Y-shaped molecule having an antigen-binding site towards the outer end of each arm. The remainder of the structure, and particularly the stem of the Y, mediates the effector functions associated with immunoglobulins.

- 25 **[0005]** Natural immunoglobulins have been used in assay, diagnosis and, to a more limited extent, therapy. However, such uses, especially in therapy, have been hindered by the polyclonal nature of natural immunoglobulins. A significant step towards the realisation of the potential of immunoglobulins as therapeutic agents was the discovery of procedures for the production of monoclonal antibodies of defined specificity [1]. However, most MAbs are produced by hybridomas which are fusions of rodent spleen cells with rodent myeloma cells. The resultant MAbs are therefore essentially rodent proteins. There are few reports of the production of human MAbs.

- 30 **[0006]** Since most available MAbs are of rodent origin, they are naturally antigenic in humans and thus can give rise to an undesirable immune response termed the HAMA (Human Anti-Mouse Antibody) response. Therefore, the use of rodent MAbs as therapeutic agents in humans is inherently limited by the fact that the human subject will mount an immunological response to the MAb and will either remove it entirely or at least reduce its effectiveness.

[0007] Therefore proposals have been made for making non-human MAbs less antigenic in humans. Such techniques can be generically termed "humanisation" techniques. These techniques generally involve the use of recombinant DNA technology to manipulate DNA sequences encoding the polypeptide chains of the antibody molecule.

- 35 **[0008]** Early methods for humanising MAbs related to production of chimeric antibodies in which an antigen binding site comprising the complete variable domains of one antibody are fused to constant domains derived from a second antibody. Methods for carrying out such chimerisation procedures are described in EP-A-0 120 694 (Celltech Limited), EP-A-0 125 023 (Genentech Inc.), EP-A-0 171 496 (Res. Dev. Corp. Japan), EP-A-0173494 (Stanford University) and EP-A-0 194 276 (Celltech Limited).

- 40 **[0009]** EP-A-0 194 276 discloses a process for preparing an antibody molecule having the variable domains from a mouse MAb and the constant domains from a human immunoglobulin. It also describes the production of an antibody molecule comprising the variable domains of a mouse MAb, the CH1 and CL domains of a human immunoglobulin and a non-immunoglobulin-derived protein in place of the Fc portion of the human immunoglobulin.

- 45 **[0010]** Subsequently, a number of further patent applications have been published relating to chimeric antibodies, including tumour specific chimeric antibodies. Among these applications are WO-A-87/02671 (Int. Gen. Eng. Inc.), EP-A-0 256 654 (Centocor), EP-A-0 266 663 (Int. Gen. Eng. Inc. & Oncogen), WO-A-89/00999 (Int. Gen. Eng. Inc.) and EP-A-0 332 424 (Hybritech Inc.).

- 50 **[0011]** Such humanised chimeric antibodies, however, still contain a significant proportion of non-human amino acid sequence, i.e. the complete variable domains. Thus, such humanised antibodies may elicit some HAMA response, particularly if administered over a prolonged period [2].

- [0012]** In an alternative approach, described in EP-A-0 239 400 (Winter), the complementarity determining regions (CDRs) of a mouse MAb have been grafted onto the framework regions of the variable domains of a human immunoglobulin by site directed mutagenesis using long oligonucleotides. Such CDR-grafted humanised antibodies are less likely to give rise to a HAMA response than humanised chimeric antibodies in view of the lower proportion of non-human amino acid sequence which they contain. There are three CDRs (CDR1, CDR2 and CDR3) in each of the heavy and light chain variable domains.

55 **[0013]** The earliest work on CDR-grafted humanised MAbs was carried out on a MAb recognising the synthetic antigen NP or NIP. However, subsequently, examples in which a mouse MAb recognising lysozyme and a rat MAb

recognising an antigen on human T cells respectively were humanised have been described [3, 4]. The preparation of the CDR-grafted antibody to the antigen on human T cells is also described in WO-A-89/07452 (Medical Research Council). Recently the preparation of a humanised CDR-grafted antibody that binds to the interleukin 2 receptor has been described [5]. Further examples of humanised CDR-grafted antibodies having specificity for anti-viral [6, 7], anti-tumour [8] and anti-T cell [9 and EP-A-0 403 156] antigens have been described more recently.

[0014] Our copending International Patent Specification No. WO-A-91/09967 relates to the CDR grafting of antibodies in general.

[0015] It has been widely suggested that immunoglobulins, and in particular MABs, could potentially be very useful in the diagnosis and treatment of cancer [10, 11]. There has therefore been much activity in trying to produce immunoglobulins or MABs directed against tumour-specific antigens. So far, over one hundred MABs directed against a variety of human carcinomas have been used in various aspects of tumour diagnosis or treatment [12].

[0016] There have been a number of papers published concerning the production of chimeric monoclonal antibodies recognising cell surface antigens. For instance, genetically engineered murine/human chimeric antibodies which retain specificity for tumour-associated antigens have been described [13 and WO-A-89/01783]. Also, a recombinant murine/human chimeric monoclonal antibody specific for common acute lymphocytic leukaemia antigen has been described [14].

SUMMARY OF THE INVENTION

[0017] We have now prepared humanised antibodies to human milk fat globule (HMFG) derived from the anti-HMFG mouse MAB CTMO1 [15].

[0018] According to the present invention, there is provided a humanised antibody molecule (HAM) having specificity for human milk fat globule (HMFG) and having an antigen binding site wherein at least one of the complementarity determining regions (CDRs) of the variable domain is derived from the mouse monoclonal antibody CTMO1 (CTMO1 MAB) and the remaining immunoglobulin-derived parts of the HAM are derived from a human immunoglobulin or an analogue thereof.

[0019] The HAM may comprise a chimeric humanised antibody or a CDR-grafted humanised antibody. When the HAM comprises a CDR-grafted humanised antibody, each heavy or light chain variable domain may comprise only one or two CTMO1-derived CDRs. Preferably, however, all three heavy and light chain CDRs are derived from CTMO1.

[0020] The CTMO1 MAB is a mouse MAB of the type IgG1-kappa raised against the membrane-associated antigen of HMFG and has been extensively studied [15]. The CTMO1 MAB has been shown to recognise breast, ovarian and non-small cell lung cancers. It has been shown to internalise rapidly into target cells. Conjugates of CTMO1 and calicheamicin display highly specific cytotoxicity against appropriate cell lines, (see USP 5053394).

[0021] High levels of the antigen recognised by the CTMO1 MAB have been detected circulating in the blood of patients suffering from breast cancer. This may have a deleterious effect on pharmacokinetics and tumour localisation *in vivo*. However, circulating antigen levels in the blood of patients suffering from ovarian cancer are lower than those in breast cancer patients. It is therefore believed that the HAM of the present invention will be of particular use in the treatment of ovarian cancer.

[0022] It is believed that the CTMO1 MAB recognises the polymorphic epithelial mucin (PEM) of HMFG. Thus, preferably, the present invention provides a HAM which recognises the PEM of HMFG.

[0023] Surprisingly, it has been found that humanising the CTMO1 MAB does not substantially adversely affect its binding activity or internalisation, and can create, particularly by CDR grafting, a HAM which has better binding and internalisation characteristics than the murine antibody (see Table 1 hereinafter). This produces a HAM which is of use in both therapy and diagnosis of certain human carcinomas, for example carcinomas of ovary, breast, uterus and lung.

[0024] Preferably, the HAM of the present invention is produced by recombinant DNA technology.

[0025] The HAM of the present invention may comprise: a complete antibody molecule, having full length heavy and light chains; a fragment thereof, such as an Fab, Fab', (Fab')₂ or Fv fragment; a single chain antibody fragment, e.g. a single chain Fv; a light chain or heavy chain monomer or dimer; or a fragment or analogue of any of these or any other molecule with the same specificity as the CTMO1 MAB.

[0026] The remaining non-CTMO1 immunoglobulin-derived parts of the HAM may be derived from a suitable human immunoglobulin. For instance, when the HAM is a CDR-grafted HAM, appropriate variable region framework sequences may be used having regard to the class or type of the CTMO1 donor antibody from which the antigen binding regions are derived. Preferably, the type of human framework used is of the same or similar class or type as the donor antibody (CTMO1 is IgG1-kappa). Advantageously, the framework is chosen to maximise or optimise homology with the donor antibody sequence, particularly at positions spatially close to or adjacent the CDRs. Examples of human frameworks which may be used to construct CDR-grafted HAMs are LAY, POM, TUR, TEI, KOL, NEWM, REI and EU [16]. KOL and NEWM are suitable for heavy chain construction. REI is suitable for light chain construction. EU is particularly suitable for both heavy chain and light chain construction. Preferably, the EU framework is used as the human frame-

work for both heavy and light chain variable domains in view of its high level of homology with the CTMO1 MAb.

[0027] The light or heavy chain variable regions of the HAM may be fused to human light or heavy chain constant domains as appropriate, (the term "heavy chain constant domains" as used herein are to be understood to include hinge regions unless specified otherwise). The human constant domains of the HAM, where present, may be selected having regard to the proposed function of the antibody, in particular the effector functions which may be required. For example, the heavy chain constant domains fused to the heavy chain variable region may be human IgA, IgG or IgM domains. Preferably human IgG domains are used. IgG1 and IgG3 isotype domains may be used when the HAM is intended for therapeutic purposes and antibody effector functions are required. Alternatively, IgG2 and IgG4 isotype domains may be used when the HAM is intended for purposes for which antibody effector functions are not required, e.g. for imaging, diagnostic or cytotoxic targeting purposes. Light chain human constant domains which may be fused to the light chain variable region include human Lambda or, especially, human Kappa chains.

[0028] Analogues of human constant domains may alternatively be advantageously used. These include those constant domains containing one or more additional amino acids than the corresponding human domain, or those constant domains wherein one or more existing amino acids of the corresponding human domain has been deleted or altered. Such domains may be obtained, for example, by oligonucleotide directed mutagenesis. In the present invention, a particularly useful analogue of a heavy chain constant domain is an IgG4 constant domain in which a serine residue at position 241 of the corresponding naturally occurring human domain has been altered to a proline residue.

[0029] The remainder of the HAM need not comprise only protein sequences from human immunoglobulins. For instance, a gene may be constructed in which a DNA sequence encoding part of a human immunoglobulin chain is fused to a DNA sequence encoding the amino acid sequences of a polypeptide effector or reporter molecule.

[0030] According to a second aspect of the present invention, there is provided a process for producing the HAM of the first aspect of the invention, which process comprises:

(a) producing in an expression vector an operon having a DNA sequence which encodes an antibody heavy or light chain comprising a variable domain wherein at least one of the CDRs of the variable domain is derived from the CTMO1 MAb and the remaining immunoglobulin-derived parts of the antibody chain are derived from a human immunoglobulin;

(b) producing in an expression vector an operon having a DNA sequence which encodes a complementary antibody light or heavy chain comprising a variable domain wherein at least one of the CDRs of the variable domain is derived from the CTMO1 MAb and the remaining immunoglobulin-derived parts of the antibody chain are derived from a human immunoglobulin;

(c) transfecting a host cell with both operons; and

(d) culturing the transfected cell line to produce the HAM.

[0031] The cell line may be transfected with two vectors, the first vector containing the operon encoding the light chain-derived polypeptide and the second vector containing the operon encoding the heavy chain-derived polypeptide. Preferably, the vectors are identical except in so far as the coding sequences and selectable markers are concerned so as to ensure as far as possible that each polypeptide chain is equally expressed.

[0032] Alternatively, a single vector may be used, the vector including the operons encoding both light chain- and heavy chain-derived polypeptides.

[0033] In further aspects, the invention also includes DNA sequences coding for the heavy and light chains of the HAM of the present invention, cloning and expression vectors containing these DNA sequences, host cells transformed with these DNA sequences and processes for producing the heavy or light chains and antibody molecules comprising expressing these DNA sequences in a transformed host cell.

[0034] The general methods by which the vectors may be constructed, transfection methods and culture methods are well known per se [17, 18].

[0035] The DNA sequences which encode the CTMO1 heavy and light chain variable domain amino acid sequences (and the corresponding deduced amino acid sequences) are given hereinafter in the sequence listing as Sequence ID No. 1 and Sequence ID No. 2 respectively.

[0036] DNA coding for human immunoglobulin sequences may be obtained in any appropriate way. For example, amino acid sequences of preferred human acceptor frameworks, such as LAY, POM, KOL, REI, EU, TUR, TEI and NEWM, are widely available to workers in the art. Corresponding DNA sequences which code for these amino acid sequences may be inferred or deduced by reverse application of the genetic code. Similarly, the amino acid sequences of human constant region domains are well known and DNA sequences which code for them may be readily deduced.

[0037] The standard techniques of molecular biology may be used to prepare DNA sequences coding for CDR-

grafted products. Desired DNA sequences may be synthesised completely or in part using oligonucleotide synthesis techniques. Site-directed mutagenesis and polymerase chain reaction (PCR) techniques may be used as appropriate. For example, oligonucleotide directed synthesis [19] may be used. Also, oligonucleotide directed mutagenesis of a pre-existing variable domain region [3, 4] may be used. Enzymatic filling-in of gapped oligonucleotides using T4 DNA polymerase [5] may be used.

[0038] Any suitable host cell/vector system may be used for expression of the DNA sequences coding for the chimeric or CDR-grafted heavy and light chains. Bacterial, e.g. *E. coli*, and other microbial systems may be used, in particular for expression of antibody fragments, e.g. Fv, Fab and Fab' fragments and single chain antibody fragments, e.g. single chain Fvs. Eucaryotic, e.g. mammalian host cell, expression systems may be used for production of larger chimeric or CDR-grafted antibody products, including complete antibody molecules. Suitable mammalian host cells include CHO cells and myeloma or hybridoma cell lines, for example NSO cells.

[0039] The present invention also includes therapeutic and diagnostic compositions containing the HAM of the invention, particularly a conjugate molecule comprising a HAM conjugated to an effector or reporter molecule and uses of such compositions in therapy and diagnosis. Such therapeutic and diagnostic compositions typically comprise a HAM according to the invention together with a pharmaceutically acceptable excipient, diluent or carrier, e.g. for *in vivo* use.

[0040] Therapeutic and diagnostic uses typically comprise administering a pharmaceutically effective amount of a HAM according to the invention to a human subject. The exact dose to be administered will vary according to the intended use of the HAM and on the age and condition of the patient but may be typically varied from about 0.1 mg to 1000mg, for example from about 1mg to 500 mg. The HAM may be administered as a single dose, or in a continuous manner over a period of time. Doses may be repeated as appropriate. The HAM may be formulated in accordance with conventional practice for administration by any suitable route, and may generally be in a liquid form [e.g. a solution of the antibody in a sterile physiologically acceptable buffer] for administration by for example an intravenous, intraperitoneal or intramuscular route.

[0041] In the HAM of the first aspect of the invention and the process of the second aspect of the invention, the heavy and light chain variable domains of the HAM may comprise either the entire variable domains of the CTMO1 MAb or may comprise framework regions of a human variable domain having grafted thereon one, two or all three of the CDRs of the CTMO1 MAb. Thus, the HAM may comprise a chimeric humanised antibody or a CDR-grafted humanised antibody.

[0042] When the HAM is a CDR-grafted humanised antibody, in addition to the CDRs, specific variable region framework residues may be altered to correspond to non-human, i.e. the CTMO1 mouse, residues. Preferably, the CDR-grafted humanised antibodies of the present invention include CDR-grafted humanised antibodies as defined in our International Patent Specification WO-A-91/09967. The disclosure of WO-A-91/09967 is incorporated herein by reference.

[0043] Preferably, the CDRs of the light chain correspond to the Kabat CTMO1 MAb CDRs at CDR1 (residues 24-34) and CDR2 (residues 50-56) and to the structural loop residues (residues 91-96) or Kabat CTMO1 MAb CDR residues (residues 89-97) in CDR3. (The residue designations given above and elsewhere in the present application are numbered according to the Kabat numbering system [16]). In addition, the light chain may have mouse CTMO1 residues at one or more of residues 1, 2, 3, 36, 37, 45, 48, 49, 60, 63, 70, 84, 85, 87 and 108. In preferred embodiments, when the human framework used is EU, the light chain comprises Kabat CTMO1 MAb CDRs at all of CDR1, CDR2 and CDR3 and preferably additional CTMO1 residues at positions 3, 36, 37, 45, 48, 63 and 108, or especially additional CTMO1 residues at positions 3, 36, 63 and 108 only.

[0044] Preferably, the CDRs of the heavy chain correspond to the Kabat CTMO1 MAb CDRs at all of CDR1 (26 to 35), CDR2 (50 to 65) and CDR3 (95 to 102). In addition, the heavy chain may have mouse CTMO1 residues at one or more of residues, 2, 6, 23, 37, 48, 49, 67, 69, 73, 76, 78, 80, 88, 91 and 94. In particularly preferred embodiments, when the human framework used is EU, the heavy chain framework comprises additional CTMO1 MAb residues at positions 2, 37, 71 and 73, and especially in addition at positions 48, 67 and 69.

[0045] In addition, EU has a particularly idiosyncratic J region between residues 103 to 113 and it may be useful to include the murine amino acids, a consensus human J region or a suitable combination of both at residues 103 to 108 inclusive. When the EU framework is used, preferably heavy chain residues 94, 103, 104, 105 and 107 are murine residues, since in the case of these residues, the murine sequence is more frequently found in human VH sequences than the EU residues.

BRIEF DESCRIPTION OF THE DRAWINGS

[0046] The present invention is now described, by way of example only, with reference to the accompanying drawings, in which:

Figure 1 is a schematic diagram of plasmid pRR62;
 Figure 2 is a schematic diagram of plasmid pAL41;
 Figure 3 is a schematic diagram of plasmid pMRR017;
 Figure 4 is a schematic diagram of plasmid pHMC34;
 5 Figure 5 is a schematic diagram of plasmid pMRR011;
 Figure 6 is a schematic diagram of plasmid pHMC32;
 Figure 7 is a schematic diagram of plasmid pMRR022;
 Figure 8 is a schematic diagram of plasmid pMRR014;
 Figure 9 is a schematic diagram of plasmid pHMC33;
 10 Figure 10 is a schematic diagram of plasmid pMRR001;
 Figure 11 is a schematic diagram of plasmid pHMC35;
 Figure 12 is a schematic diagram of plasmid pHMC38;
 Figure 13 is a schematic diagram of plasmid pHMC40;
 Figure 14 is a schematic diagram of plasmid pHMC41;
 15 Figure 15 is a schematic diagram of plasmid pHMC42;
 Figure 16 shows the alignment of oligonucleotides H1 to H8 in the formation of the gH1 coding sequence;
 Figure 17 is a schematic diagram of plasmid pAL51;
 Figure 18 is a schematic diagram of plasmid pAL52;
 Figure 19 is a schematic diagram of plasmid pMRR010;
 20 Figure 20 is a schematic diagram of plasmid pAL47;
 Figure 21 is a schematic diagram of plasmid pAL48;
 Figure 22 is a graph of a direct binding ELISA on transiently expressed chimeric antibodies;
 Figure 23 is a graph of a direct binding ELISA on transiently expressed CDR-grafted antibodies; and
 Figure 24 is a graph of a competition EIA on transiently expressed chimeric and CDR-grafted antibodies.
 25 Figure 25 is a graph comparing the effects on tumour size obtained by treating nude mice implanted with a human ovarian xenograft tumour with a humanised CDR grafted CTMO1 and a murine CTMO1 antibody each conjugated to the hydroxysuccinimide derivative of 4-mercapto-4-methylpentanoic acid disulphide of N-acetyl calicheamicin γ^1 .

30 DESCRIPTION OF SPECIFIC EMBODIMENTS

[0047] The following description of certain embodiments of the invention is provided by way of example only and is not to be regarded as placing any limitation on the scope of the protection claimed.

35 MOLECULAR CLONING AND CONSTRUCTION OF THE CTMO1 CHIMERIC HEAVY CHAIN

[0048] The heavy chain variable domain of CTMO1 was cloned using the polymerase chain reaction. This enabled the construction of the chimeric version in a single step as described below.

40 [0049] Polyadenylated RNA was isolated from the CTMO1 hybridoma cell line using the guanidinium isothiocyanate/lithium chloride method [17]. Double stranded cDNA was synthesised and used as a template for PCR amplification of the VH gene. A set of twenty four 5' forward primers were synthesised to complement a sequence within the murine leader sequence of VH domains [16] and to introduce a BstEII restriction site. A set of twelve 3' reverse primers was synthesised to complement the framework 4 region of VH [20] and included an Apal restriction site.

45 [0050] The sequence of the basic 5' primer is given in the Sequence Listing as ID No. 3. The set of twenty four primers was based on this primer as follows. In one group of twelve primers, residue 27 remained as C. In three subgroups of four primers, residue 25 either remained as G or is altered to C or T. In each subgroup, the four primers differed at residue 28, which was A, C, G or T. In the subgroups where residue 25 is C or T, the sixth amino acid is His.

50 [0051] In the second group of twelve primers, residue 27 is changed to G. In three subgroups of four primers, residue 25 either remains as G or is altered to C or T. In each subgroup, the four primers differed at residue 28, which was A, C, G or T. Where residue 25 is C or G, amino acid 6 is Gln and where residue 25 is T, amino acid residue 6 is His. Where residue 28 is T or C, amino acid residue 7 is Cys. Where residue 28 is G, amino acid residue 7 is Trp.

[0052] The sequence of the basic 3' PCR primer is given in the Sequence Listing as ID No. 4. The set of twelve primers was based on this primer as follows. Residue 5 could remain as G or could be altered to A or T. Residue 11 either remains as A or is altered to G. Residue 12 either remains as A or is altered to C.

55 [0053] PCR amplification of CTMO1 VH was carried out using the following conditions:

[0054] 10 pmol each primer; 20 ng cDNA; 0.5 U Taq polymerase; 94°C 1 min; 50 °C 2 min; 72°C 3 min; for 40 cycles.

[0055] The PCR amplified VH fragment was restricted with BstEII and Apal and ligated to an adaptor to reconstruct the leader sequence and add a 5' HindIII restriction enzyme site. The sequence of the adaptor used is given in the

Sequence Listing as Sequence ID No. 5 and codes in part for the leader amino acid sequence of the VH domain of the murine anti TAG-72 monoclonal antibody B72.3 (WO-A-89/01783).

[0056] The adapted fragment was then cloned into the HindIII/ApaI sites of the vector pE1004 to give plasmid pRR62 shown in Figure 1. Plasmid pRR62 consists of an SV40 origin of replication followed by the hCMV-MIE promoter/enhancer region. The promoter/enhancer controls a nucleotide sequence encoding a chimeric heavy chain comprising the CTMO1 heavy chain variable domain fused to human g4 constant domains. Downstream of the coding sequence is a poly A site and gpt gene.

[0057] The heavy chain variable region of several independent clones of pRR62 were sequenced. The DNA sequence and deduced amino sequence for CTMO1 VH are given in Sequence ID No. 1.

MOLECULAR CLONING AND CONSTRUCTION OF THE CTMO1 CHIMERIC LIGHT CHAIN

[0058] Polyadenylated RNA was isolated from the CTMO1 hybridoma cell line using the guanidinium isothiocyanate/lithium chloride method [17]. Double stranded cDNA was synthesised [21] and a cDNA library was constructed in plasmid pSP64 [22] using EcoRI linkers. A screening probe was synthesised, complementary to mouse immunoglobulin light chain constant region by PCR amplification. The light chain probe was a 318 bp PCR fragment encoding the mouse kappa light chain constant region [23].

[0059] The probe was radio-labelled ($g^{32}P$) ATP by random hexanucleotide priming and was used to screen the cDNA library.

[0060] The clone which encoded the complete leader, variable and constant domains of light chain was isolated and designated as pRB63.

[0061] A fragment of pRB63, which encodes the variable domain of the light chain was recovered by PCR amplification. The PCR primers introduced a BstBI and SphI restriction sites at the 5' and 3' ends of the VL region respectively to enable subsequent cloning of the fragment.

[0062] The PCR amplified fragment of plasmid pRB63 was restricted with BstBI/SphI and ligated between the BstBI/SphI sites of plasmid pMRR010 to produce plasmid pAL41, which is shown in Figure 2. Plasmid pAL41 consists of a glutamine synthetase cDNA having downstream of it the hCMV-MIE promoter/enhancer region. The promoter/enhancer region controls a nucleotide sequence encoding a chimeric light chain comprising the CTMO1 light chain variable domain fused to a human CK constant domain. Downstream of the coding sequence is a poly A site.

[0063] Nucleotide sequence analysis was carried out according to the chain termination procedure [24]. The VH coding sequence insert in pRR62 and the VL coding sequence insert in pAL41 were fully sequenced. The DNA and predicted amino acid sequences for the unprocessed variable domains of the CTMO1 heavy and light chains are shown in the Sequence Listing appended to the end of the description as Sequence ID No. 1 and No. 2 respectively.

[0064] Sequence No. 1 shows the sequence coding for the VH domain and the predicted amino acid sequence. The leader sequence for the heavy chain runs from residue 1 to residue 19 as shown in Sequence No. 1. Sequence No. 2 shows the sequence coding for the VL domain together with the predicted amino acid sequence. The leader sequence for the light chain runs from residue 1 to residue 20 as shown in Sequence No. 2. Examination of the derived amino acid sequences revealed considerable homology with other characterised immunoglobulin genes. The CTMO1 MAB was confirmed to be an IgG1-kappa antibody.

PREPARATION OF CHIMERIC ANTIBODY PRODUCTS

CHIMERIC LIGHT CHAIN VECTOR

[0065] A ClaI-EcoRI fragment of pAL41 carrying the hCMV promoter and chimeric light chain was cloned into plasmid pMRR017 which is shown in Figure 3. Plasmid pMRR017 has a GS mini gene (WO-A-87/04462), hCMV-MIE promoter/enhancer region, a polylinker sequence and a poly A site. This produced plasmid pHMC34, which is shown in Figure 4. In plasmid pHMC34, the chimeric light chain gene is under the control of the hCMV-MIE promoter/enhancer sequence.

CHIMERIC HEAVY CHAIN VECTORS

IgG1 CONSTRUCT

[0066] A HindIII-ApaI fragment containing the sequence encoding the VH domain was excised from plasmid pRR62 (Figure 1). This fragment was inserted between the HindIII and ApaI sites of plasmid pMRR011. Plasmid pMRR011 is shown in Figure 5 and comprises an hCMV-MIE promoter/enhancer region, an SV40 polyadenylation sequence, a gpt gene and a sequence encoding a human IgG1 heavy chain lacking a variable domain. The plasmid thus produced,

pHMC32, is shown in Figure 6 and has a chimeric heavy chain coding sequence under the control of the hCMV-MIE promoter/enhancer. The chimeric heavy chain has the VH domain from the CTM01 MAb fused to human IgG1 constant domains.

5 IgG2 CONSTRUCT

[0067] The HindIII-ApaI fragment of pRR62 (Figure 1) was inserted between the HindIII and ApaI sites of a plasmid containing an hCMV-MIE promoter, a polylinker site and a nucleotide coding sequence which encodes the three constant domains of a human IgG2 antibody. This yielded plasmid pMRR022 which encodes a chimeric heavy chain having the CTM01 variable domain linked to the human IgG2 constant domains.

IgG4 CONSTRUCT

[0068] The HindIII-ApaI fragment of pRR62 (Figure 1) was inserted between the HindIII and ApaI sites of plasmid pMRR014 to produce plasmid pHMC33. Plasmids pMRR014 and pHMC33 are shown in Figure 8 and 9 respectively. Plasmid pMRR014 has an hCMV-MIE promoter, a polylinker site and a nucleotide coding sequence which encodes the three constant domains of a human IgG4 antibody. Plasmid pHMC33 is identical to plasmid pHMC32 except that the coding sequence encodes a chimeric heavy chain having the CTM01 variable domain and human IgG4 constant domains in place of the human IgG1 constant domains.

ALTERED IgG4 CONSTRUCT

[0069] The HindIII-ApaI fragment was reisolated from plasmid pHMC33. Plasmid pMRR001 shown in Figure 10 was digested with HindIII and ApaI. The large fragment was isolated and ligated to the HindIII-ApaI fragment of pHMC33 to produce plasmid pHMC35, shown in Figure 11. Plasmid pHMC35 is almost identical to plasmid pHMC32 except that the coding sequence encodes a chimeric heavy chain having the CTM01 variable domain and altered human IgG4 (hereinafter referred to as IgG4P) constant domains in place of the human IgG1 constant domains.

[0070] The alteration in the constant domains comprises a change of a serine residue in the hinge region at position 241 to a proline residue. This change advantageously abolished the formation of an 80 KD half antibody which otherwise occasionally is formed with IgG4 constant domains.

CHIMERIC HEAVY AND LIGHT CHAIN VECTORS

[0071] Vectors were constructed having operons coding for both heavy and light chains within the same vector.

[0072] A NotI-SalI fragment carrying the hCMV-MIE promoter/enhancer, the chimeric light chain encoding sequence and the SV40 poly A site together with the GS mini gene was excised from plasmid pHMC34 (Figure 4). A NotI-HindIII fragment carrying the hCMV-MIE promoter/enhancer was excised from plasmid pHMC35 (Figure 11). A HindIII-SalI fragment carrying the altered IgG4 heavy chain coding sequence and SV40 poly A site was excised from plasmid pHMC35 (Figure 11). These three fragments were ligated together to produce plasmid pHMC38, which is shown in Figure 12, and codes for expression of chimeric light chain together with the altered IgG4 chimeric heavy chain.

[0073] Plasmids pHMC32, pMRR022 and pHMC33 were digested with HindIII and EcoRI and the fragments containing the chimeric heavy chain encoding sequences were isolated. The isolated fragments were each ligated with the large HindIII-SalI fragment of pHMC38 (Figure 12) and an EcoRI-SalI fragment comprising the SV40 poly A region. The ligations produced plasmids pHMC40, pHMC41 and pHMC42 (shown in Figures 13 to 15 respectively). pHMC40 encodes a heavy chain having IgG1 constant domains. pHMC41 encodes IgG2 constant domains and pHMC42 encodes IgG4 constant domains.

PREPARATION OF CDR-GRAFTED ANTIBODY PRODUCTS

[0074] It was decided to use the EU human antibody framework [16] for carrying out the CDR-grafting. The strategy followed for CDR-grafting was as set out in our International Patent Specification No. WO-A-91/09967.

[0075] Two CDR-grafted heavy chains were designed. In the first, gH1, all three CDRs [as defined by Kabat, ref. 16] were changed to murine residues. In addition, residues 2, 37, 71, 73, 94, 103, 104, 105 and 107, which are outside the Kabat CDRs, were also changed to murine residues. In the second, gH2, in addition to those murine residues in gH1, residues 48, 67 and 69 were changed to murine residues with a view to improving packing of the VH domain.

[0076] Two CDR-grafted light chains were also designed. In the first, gL1, all three CDRs [as defined by Kabat, ref. 16] were changed to murine residues. In addition residues 3, 36, 63 and 108, which are outside the Kabat CDRs, were changed to murine residues. In the second, gL2, in addition to those murine residues in gL1, residues 37, 45 and 48

were changed to murine residues with a view to improving packing.

[0077] A nucleotide sequence coding for the gH1 variable domain was produced by oligonucleotide assembly using oligonucleotides H1 to H8. The sequences for these oligonucleotides are given in the Sequence Listing at the end of the description under Sequence ID Nos. 6 to 13. The way in which these oligonucleotides are assembled to produce the gH1 coding sequence is shown in Figure 16. The amino acid sequence coded for by this gH1 sequence is shown in the sequence listing under Sequence ID No. 14.

[0078] A nucleotide sequence coding for the gH2 variable domain was also produced by oligonucleotide assembly using oligonucleotides H1, H2, H3A, H4, H5, H6A, H7 and H8. Oligonucleotide H3A differs from oligonucleotide H3 (Sequence ID No. 8) in that residues 55 to 57 have been changed from GTG to GCA and residues 61 to 63 have been changed from ATT to CTG. Oligonucleotide H6A differs from oligonucleotide H6 (Sequence ID No. 11) in that residues 70 to 72 have been changed from TAC to TAA. Thus, the gH2 variable domain encodes the same sequence as is shown under Sequence ID No. 14, except that at residue 67, MET has been changed to ILE; at residue 87, VAL has been changed to ALA; and at residue 89, ILE has been changed to LEU.

[0079] A nucleotide sequence coding for the gL1 variable domain was produced by oligonucleotide assembly using oligonucleotides L1 to L8. The sequences for these oligonucleotides are given in the Sequence Listing at the end of the description under Sequence ID Nos. 15 to 22. The way in which these nucleotides are assembled is similar to that shown in Figure 16 for the gH1 coding sequence (except that L is substituted for H). The amino acid sequence coded for by the assembled gL1 variable domain coding sequence is shown in the Sequence Listing under Sequence ID No. 20.

[0080] A nucleotide sequence coding for the gL2 variable domain was produced by oligonucleotide assembly using oligonucleotides L1, L2A, L3A and L4 to L8. Oligonucleotide L2A differs from oligonucleotide L2 (Sequence ID No. 16) in that residues 28 to 30 have been changed from CAG to GTA. Oligonucleotide L3A differs from oligonucleotide L3 (Sequence ID No. 17) in that residues 25 - 27 have been changed from CAG to CTC, residues 49 - 52 have been changed from AAG to CAG and residues 59 - 61 have been changed from CAT to ATC. Thus, the gL2 variable domain encodes the same sequence as is shown under Sequence ID NO. 23, except that: at residue 23, Gln has been changed to Val; at residue 62, Gln has been changed to Leu; at residue 60, Lys has been changed to Gln; and at residue 73, Met has been changed to Ile.

[0081] For gene assembly 1 pmol of H2 - H7 or L2 - L7 was mixed with 10 pmol of H1 and H8 or L1 and L8 in a 100 ml reaction with 5U Taq polymerase. A PCR reaction was done using 30 cycles (95°C, 1 min. 50°C 1 min; 72°C 1 min). The resulting fragments were cut with HindIII and Apal for VL with Bstb1 and SP11 for VH.

[0082] The nucleotide sequences coding for gH1 and gH2 were cloned as HindIII-Apal fragments into plasmid pMRR014 (Figure 8) to produce plasmids pAL51 and pAL52 (Figure 17 and 18 respectively).

[0083] The nucleotide sequences coding for gL1 and gL2 were cloned as HindIII-Apal fragments into plasmid pMRR010 (Figure 19) to produce plasmids pAL47 and pAL48 (Figures 20 and 21 respectively).

TRANSIENT EXPRESSION OF CHIMERIC/CHIMERIC OR CDR-GRAFTED/CHIMERIC ANTIBODIES

[0084] The following plasmids:
pHMC38, pHMC40, pHMC41 and pHMC42

and the following pairs of plasmids:
pAL47, pHMC33; pAL48, pHMC33; pAL51, pAL41; pAL52,
pAL41; and pAL48, pAL41;

were each transfected or cotransfected into CHO-L761h cells for transient expression.

[0085] Assembly ELISA assays on culture supernatants resulting from the single transfected cells showed that they contained assembled antibody.

[0086] The assembly ELISA assay for quantifying antibody yields used microwell plates coated with a goat F(ab')₂ anti-human IgGf. Following incubation with transfected culture supernatants, bound chimeric or CDR-grafted antibody was revealed with a horseradish peroxidase (HRP)-conjugated murine anti-human IgK antibody using tetramethyl benzidine (TMB) as the substrate. Concentrations of chimeric or CDR-grafted whole antibody in the samples were interpolated from a calibration curve generated from serial dilutions of purified chimeric B72.3 γ4 antibody [25].

BINDING ACTIVITY OF TRANSIENTLY EXPRESSED CHIMERIC OR CDR-GRAFTED ANTIBODIES

[0087] Direct binding ELISA assays for determining the binding activity of the transiently expressed antibodies were carried out as follows.

[0088] An affinity column was prepared by attaching the CTM01 MAb to a suitable chromatographic medium in conventional manner. In a first method, pooled human urine samples were applied directly to the affinity column. In a second method, human milk was subjected to low speed centrifugation to separate the cream from skimmed milk. The

skimmed milk was then subjected to high speed centrifugation to produce an aqueous and a lipid component. The aqueous component was applied to the affinity column.

[0089] Once the affinity column was loaded, by either of the two methods, column fractions were eluted at high and low pHs, neutralised and assayed for reactivity with the CTM01 MAb. Fractions showing reactivity were pooled and dialysed. The pooled fractions contained the polymorphic epithelial mucin (PEM) recognised by the CTM01 MAb.

[0090] Microwell plates were coated with PEM obtained as described above. The microwells were then incubated with serial dilutions of culture supernatants. Binding of chimeric or CDR-grafted antibody was revealed and quantified by use of an HRP-conjugated murine anti-human IgK antibody.

[0091] The results of direct binding ELISA assays on the supernatants from singly transfected cells are shown in Figure 22. These assays confirm that all the supernatants contained antibodies capable of binding to PEM. No significant differences in binding activity were observed.

[0092] The direct binding ELISA assays on the supernatants from doubly transfected cells confirmed that the supernatants contained antibodies capable of binding to PEM and that the chimeric/chimeric antibody bound better than any of the CDR-grafted/chimeric antibodies.

[0093] A competition binding assay was carried out using polystyrene beads coated with PEM obtained as described above. CTM01 MAb was radiolabelled with ¹²⁵I and was used to compete with the antibody produced by the pHMC40 (IgG1) transfected cells. The potency of the chimeric antibody was 84-102% that of the CTM01 MAb.

TRANSIENT EXPRESSION OF CDR-GRAFTED/CDR-GRAFTED ANTIBODIES

[0094] The following pairs of plasmids:

pAL47, pAL51; pAL47, pAL52; pAL48, pAL51; and pAL48, pAL52;

were cotransfected into CHO-L761 cells.

[0095] Direct binding assays were carried out on the culture supernatants produced by the doubly transfected cell lines.

[0096] The results of these assays are shown in Figure 23, together with some results for chimeric/CDR-grafted antibodies.

[0097] From all the direct binding assays referred to above, it can be determined that the order of binding activity of the various antibodies produced by transient expression is as follows:

$$cLcH3 > gL1ch = gL1gH2 > cLgH2 = gL2H2 = gL1gH1 = gL2cH > gL2gH1.$$

(wherein: cL = chimeric light chain;

cH = chimeric heavy chain

gL1 = CDR-grafted light chain with lowest number of amino acid changes;

gL2 = CDR-grafted light chain with highest number of amino acid changes;

gH1 = CDR-grafted heavy chain with lowest number of amino acid changes;

gH2 = CDR-grafted heavy chain with highest number of amino acid changes).

[0098] The more active variants (cLcH, gL1cH, gL1gH2 and gL2gH2) together with the CTM01 MAb were tested in a competition enzyme immunoassay (EIA). Microwell plates were coated with PEM obtained as described above. The CTM01 MAb was biotinylated and was used to compete with the four variants referred to above. Bound biotinylated CTM01 MAb was revealed and quantified using a streptavidin-HRP conjugate and TMB.

[0099] The results of the competition EIA are shown in Figure 24, which shows the same ranking of binding activity as set out above, except that the gL1cH combination shows greater activity than the cLcH combination.

[0100] It can thus be seen that chimeric, chimeric/CDR-grafted and CDR-grafted antibodies which recognise the same antigen as the CTM01 MAb have successfully been produced.

IN VITRO CELL BINDING AND INTERNALISATION OF CDR-GRAFTED CTM01 ANTIBODIES

[0101] Stable NSO cell lines expressing gL1gH2IgG2 CTM01 (hereinafter hu1:CTM01) and gL1gH2IgG4P CTM01 (hereinafter hu:CTM01) antibody variants were made by transfecting into NSO cells by electroporation double gene expression plasmids assembled by ligating the large (7.8 kbp) Not1/BamH1 fragment of pAL47 to the 2.4 kbp Not1/Apa1 fragment from pAL52 and either a 1.9Kbp BamH1/Apa1 (partial) fragment carrying the IgG2 constant domains or a 2kbp Apa1/BamH1 fragment carrying the IgG4P constant domains as appropriate.

[0102] Antibody, purified from the supernatant of each cultured cell line by protein-A sepharose chromatography was

radiolabelled (^{125}I) and incubated using a conventional continuous exposure method with either MX-1 or MCF-7 breast carcinoma cells. Radiolabelled murine CTMO1 was used in all tests as a comparison. All antibodies were incubated at $2\mu\text{g}/\text{million}$ cells. The total binding of antibodies to the cells and the peak net uptake of the antibodies by the cells was determined. The results are shown in Table 1 below. With both cell lines each CDR grafted antibody exhibited better binding and internalisation than the murine form.

TABLE 1

Antibody	Cell Line	Total Binding, 0° (molecules/cell)	Peak Net Uptake (molecules/cell)
hu1:CTMO1	MCF-7	650,000	150,000
hu:CTMO1	MCF-7	450,000	90,000
Murine CTMO1	MCF7	300,000	70,000
hu1:CTMO1	MX-1	1,200,000	150,000
hu:CTMO1	MX-1	1,100,000	150,000
Murine CTMO1	MX-1	800,000	80,000

REFERENCES

[0103]

1. Kohler & Milstein, Nature 265, 495-497, 1975
2. Begent et al, Br. J. Cancer, 62, 487, 1990
3. Verhoeven et al, Science, 293, 1534-1536, 1988
4. Riechmann et al, Nature, 332, 323, 324, 1988
5. Queen et al, Proc. Natl. Acad. Sci., USA, 86, 10029-10033, 1989 and WO-A-90/078861
6. Tempest et al, Biotechnology, 9, 266-271, 1991
7. Co et al, Proc. Natl. Acad. Sci., USA, 88, 2869-2873, 1991
8. Verhoeven et al, 1991 in Epenetos, A.A., (ed.), - "Monoclonal Antibodies: Applications in Clinical Oncology"
9. Gorman et al, Proc. Natl. Acad. Sci., USA, 88, 4181-4885, 1991
10. Ehrlich, P., Collected Studies on Immunity, 2, John Whey & Sons, New York, 1986
11. Levy & Miller, Ann. Rev. Med., 34, 107-116, 198-(?)
12. Schlom & Weeks, Important Advances in Oncology, 170-192, Wippincott, Philadelphia, 1985
13. Sahagan et al, J. Immunol., 137, 3, 1066-1074, 1986
14. Nishimura et al, Cancer Res., 47, 999-1005, 1987
15. Aboud-Pirak et al, Cancer Res., 48, 3188-3196, 1988
16. Kabat et al, Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA, 1987 and Wu, T.T. and Kabat, E.A. J. Exp. Med., 132, 211-250, 1970
17. Maniatis et al, Molecular Cloning, Cold Spring Harbour, New York, 1982
18. Primrose and Old, Principles of Gene Manipulation, Blackwell, Oxford, 1980
19. Jones et al, Nature, 54, 75-82, 1986
20. Orlandi et al, Proc. Natl. Acad. Sci., USA, 86, 3833-3837, 1989
21. Gubler and Hoffman, Gene, 25, 263-269, 1983
22. Melton et al, Nuc. Acids. Res., 12, 7035-7056, 1984
23. Max et al, J. Biol. Chem., 256, 5116-5120, 1981
24. Sanger et al, PNAS, 74, 5463-5467, 1977
25. Colcher et al, Proc. Natl. Acad. Sci., USA, 86, 3833-3837, 1989.

SEQUENCE LISTING

SEQUENCE ID NO: 1.

[0104] SEQUENCE TYPE: Nucleotide with deduced protein sequence.

[0105] SEQUENCE LENGTH: 416 bases.

[0106] STRANDEDNESS: Single.

[0107] TOPOLOGY: Linear.

[0108] MOLECULE TYPE: cDNA.

[0109] ORIGINAL SOURCE ORGANISM: Murine.

IMMEDIATE EXPERIMENTAL SOURCE

[0110] NAME OF CELL LINE: Hybridoma CTM01.

[0111] PROPERTIES: Coding sequence for variable domain of heavy chain of the CIM01 monoclonal antibody.

[0112] FEATURES: Leader sequence from residues 1 to 19.

Kabat
numbering } ATG GAA TGG AGC TGG GTC TTT CTC TTC TTC CTG TCG GTA ACC ACA GG
-19 -18 -17 -16 -15 -14 -13 -12 -11 -10 -9 -8 -7 -6 -5 -4
Met Glu Trp Ser Trp Val Phe Leu Phe Phe Leu Ser Val Thr Thr Gl

GTC CAT TGC CAG ATC CAG CTG CAG CAG TCT GGA CCT GAG CTG GTG AAG
-3 -2 -1 2 3 4 5 6 7 8 9 10 11 12 13
Val His Cys Gln Ile Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys

CCT GGG GCT TCA GTG AAG ATA TCC TGC AAG GCT TCT GGC TAC ACC TTC
14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe

CDR1

CDR1
ACT GAC TAC TAT ATA AAC TGG ATG AAG CAG AAG CCT GGA CAG GGA CTT 192
30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
Thr Asp Tyr Tyr Ile Asn Trp Met Lys Gln Lys Pro Gly Gln Gly Leu 64

GAG TGG ATT GGA TGG ATT GAT CCT GGA AGC GGT AAT ACT AAG TAC AAT 240
46 47 48 49 50 51 52 53 54 55 56 57 58 59 60
Glu Trp Ile Gly Trp Ile Asp Pro Gly Ser Gly Asn Thr Lys Tyr Asn 80

CDR2

GAG AAG TTC AAG GGC AAG GCC ACA TTG ACT GTA GAC ACA TCC TCC AGC 288
61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76
Glu Lys Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Thr Ser Ser Ser 96

ACA GCC TAC ATG CAG CTC AGC AGC CTG ACA TCT GAG GAC ACT GCT GTC 336
77 78 79 80 81 82 83 84 85 86 87 88 89
Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Thr Ala Val 112

TAT TTC TGT GCA AGA GAG AAA ACG AGC TAT TAC TAT GCT ATG GAC TAC 384
90 91 92 93 94 95 96 97 98 99 100 101 102
Tyr Phe Cys Ala Arg Glu Lys Thr Thr Tyr Tyr Tyr Ala Met Asp Tyr 128

CDR3

TGG GGT CAA GGA ACC TCA GTC ACT GTC TCC GC 416
103 104 105 106 107 108 109 110 111 112 113
Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ala 139

SEQUENCE ID NO: 2.

[0113] SEQUENCE TYPE: Nucleotide with deduced protein sequence.

[0114] SEQUENCE LENGTH: 399 bases.

[0115] STRANDEDNESS: Single.

[0116] TOPOLOGY: Linear.

[0117] MOLECULE TYPE: cDNA.

[0118] ORIGINAL SOURCE ORGANISM: Murine.

IMMEDIATE EXPERIMENTAL SOURCE

- 5 [0119] NAME OF CELL LINE: Hybridoma CTM01.
 [0120] PROPERTIES: Coding sequence for variable domain of light chain of the CIM01 monoclonal antibody.
 [0121] FEATURES: Leader sequence from residues 1 to 20.

10

15

20

25

30

35

40

45

50

55

CDR1

ATG	AGG	TGC	CTA	GCT	GAG	TTC	CTG	GGG	CTG	CTT	GTG	CTC	TGG	ATC	CCT	48
-20	-19	-18	-17	-16	-15	-14	-13	-12	-11	-10	-9	-8	-7	-6	-5	
Met	Arg	Cys	Leu	Ala	Gly	Phe	Leu	Gly	Leu	Leu	Val	Leu	Tyr	Ile	Pro	16
GGA	GCC	ATT	GGG	GAT	ATT	GTG	ATG	ACT	CAG	GCT	GCA	CCC	TCT	GTT	CCT	96
-4	-3	-2	-1		2	3	4	5	6	7	8	9	10	11	12	
Gly	Ala	Ile	Gly	Asp	Ile	Val	Met	Thr	Gln	Ala	Ala	Pro	Ser	Val	Pro	32
GTC	ACT	CCT	GGA	GAG	TCA	TTA	TCC	ATT	TCC	TGC	AGG	TCT	AGT	AAG	AGT	144
13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	
Val	Thr	Pro	Gly	Gly	Ser	Leu	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Lys	Ser	48
CTC	CTT	CAT	AGT	AAT	GGC	GAC	ACT	TTC	TTG	TAT	TGG	TTC	CTG	CAG	AGG	192
29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	
Leu	Leu	His	Ser	Asn	Gly	Asp	Thr	Phe	Leu	Tyr	Tyr	Phe	Leu	Gln	Arg	64

5		CCA	GCC	CAG	TCT	CCT	CAA	CTC	CTG	ATA	TAT	CGG	ATG	TCC	AAC	CTT	GCC	240
		40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	80
		Pro	Gly	Gln	Ser	Pro	Gln	Leu	Leu	Ile	Tyr	Arg	Met	Ser	Asn	Leu	Ala	
	CDR2																	
		TCC	GGA	GTC	CCA	GAC	AGG	TTC	AGT	GGC	AGT	GGG	TCA	GGA	ACT	GCT	TTC	288
		56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	96
		Ser	Gly	Val	Pro	Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Ala	Phe	
10																		
		ACA	CTG	AGA	GTC	AGT	AGA	CTG	GAG	GCT	GAG	GAT	CTG	GCT	GTT	TAT	TAC	336
		72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	112
		Thr	Leu	Arg	Val	Ser	Arg	Val	Glu	Ala	Gly	Asp	Phe	Gly	Val	Tyr	Tyr	
	CDR3																	
		TGT	ATG	CAA	CAT	CTA	GAA	TAT	CCT	TTC	ACG	TTC	GGT	GCT	GGG	ACC	AAG	384
		88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	128
		Cys	Met	Gln	His	Leu	Gly	Tyr	Pro	Phe	Ser	Phe	Gly	Ala	Gly	Thr	Lys	
		CTG	GAG	CTG	AAA	CGG												399
		104	105	106	107	108												133
		Leu	Gly	Leu	Lys	Arg												
20																		

SEQUENCE ID NO. 3

- 25 [0122] SEQUENCE TYPE: Nucleotide sequence with corresponding amino acid sequence
 [0123] SEQUENCE LENGTH: 28 bases
 [0124] STRANDEDNESS: Single
 [0125] TOPOLOGY: Linear
 [0126] MOLECULAR TYPE: Synthesised DNA
 30 [0127] PROPERTIES: 5' forward primers for PCR amplification of murine VH domains
 [0128] FEATURES: Complementary to leader sequence of murine VH domains with introduced BstEII restriction site at residues 7 to 13

35	GGTGGCG GTA ACC ACA GGT GTC CAG TCA	28
	Val Thr Thr Gly Val Gln Ser	7

SEQUENCE ID NO. 4

- 40 [0129] SEQUENCE TYPE: Nucleotide sequence
 [0130] SEQUENCE LENGTH: 36 bases
 [0131] STRANDEDNESS: Single
 [0132] TOPOLOGY: Linear
 [0133] MOLECULE TYPE: Synthesised DNA
 45 [0134] PROPERTIES: 3' reverse primer for PCR amplification of murine VH domains
 [0135] FEATURES: Complementary to framework 4 region of murine VH including an Apal restriction site at residues 25 to 30

50	AGTGGCAGAG AAGTCGGAGT TGCTTCCCGG GTAGAC	36
----	---	----

SEQUENCE ID NO. 5

- 55 [0136] SEQUENCE TYPE: Nucleotide sequences with corresponding amino acid sequence
 [0137] SEQUENCE LENGTH: 50 bases (sense strand) and 51 bases (anti-sense strand)
 [0138] STRANDEDNESS: Double
 [0139] TOPOLOGY: Linear

EP 0 781 845 B1

[0140] MOLECULE TYPE: Synthesised DNA
 [0141] PROPERTIES: Leader sequence adaptor for murine VH domain
 [0142] FEATURES: Comprises a HindIII-BstEII fragment in part coding for the leader sequence of the VH domain of murine monoclonal antibody B72.3

5

```

AGCTTGCCGC CACC ATG GAA TGG AGC TGG GTC TTT CTC TTC TTC CTG TCG      50
ACGGCG GTGG TAC CTT ACC TCG ACC CAG AAA GAG AAG AAG GAC AGC CATTG      51
Met Glu Trp Ser Trp Val Phe Leu Phe Phe Leu Ser      12
  
```

10

SEQUENCE ID NO: 6

[0143] SEQUENCE TYPE: Nucleotide.

15

SEQUENCE LENGTH: 21.

[0144] STRANDEDNESS: Single.

[0145] TOPOLOGY: Linear.

20

[0146] MOLECULE TYPE: Synthetic oligonucleotide

[0147] PROPERTIES: Used for assembly of CDR-grafted heavy chain.

[0148] FEATURES: HindIII site at residues 7-12

25

```

GCGGCAAGC TTGCCGCCAC C      21
  
```

SEQUENCE ID NO: 7.

30

[0149] SEQUENCE TYPE: Nucleotide

SEQUENCE LENGTH: 96.

[0150] STRANDEDNESS: Single.

35

[0151] TOPOLOGY: Linear.

[0152] MOLECULE TYPE: Synthetic oligonucleotide.

[0153] PROPERTIES: Used for assembly of CDR-grafted heavy chain.

40

```

TCTCAGATTC AGCTGGTGCA GTCTGGAGCA GAGGTGAAGA AGCCTGGATC      50
TTCTGTGAAG GTGTCTTGTA AGGCATCTGG ATACACCTTC ACCGAC      96
  
```

SEQUENCE ID NO: 8.

45

[0154] SEQUENCE TYPE: Nucleotide.

SEQUENCE LENGTH: 96.

[0155] STRANDEDNESS: Single.

[0156] TOPOLOGY: Linear.

[0157] MOLECULE TYPE: Synthetic oligonucleotide.

[0158] PROPERTIES: Used for assembly of CDR-grafted heavy chain.

55

```

TGGATTGACC CTGGATCTGG AAATACAAAG TACAATGAGA AGTTCAAGGG      50
AAGAGTGACA ATTACAGTGG ACACATCCAC GAATACCGCC TACATG      96
  
```

EP 0 781 845 B1

SEQUENCE ID NO: 9.

[0159] SEQUENCE TYPE: Nucleotide.

5 SEQUENCE LENGTH: 89.

[0160] STRANDEDNESS: Single.

[0161] TOPOLOGY: Linear.

[0162] MOLECULE TYPE: Synthetic oligonucleotide.

10 [0163] PROPERTIES: Used for assembly of CDR-grafted heavy chain.

[0164] FEATURES: Apal site at residues 78-83

15 GAGAAGACCA CCTACTACTA CGCAATGGAC TACTGGGGAC AGGGAACACT 50
GGTGACAGTG TCTTCTGCCT CAACGAAGGG CCGCGCGCG 89

SEQUENCE ID NO: 10.

20 [0165] SEQUENCE TYPE: Nucleotide.

SEQUENCE LENGTH: 96.

[0166] STRANDEDNESS: Single.

25 [0167] TOPOLOGY: Linear.

[0168] MOLECULE TYPE: Synthetic oligonucleotide.

[0169] PROPERTIES: Used for assembly of CDR-grafted heavy chain.

30 CTGCACCAGC TGAATCTGAG AATGGACTCC TGTAGTTACT GACAGGAAGA 50
AGAGAAAGAC CCAGCTCCAT TCCATGGTGG CGGCAAGCTT GCGCGC 96

SEQUENCE ID NO: 11.

35 [0170] SEQUENCE TYPE: Nucleotide.

SEQUENCE LENGTH: 96.

40 [0171] STRANDEDNESS: Single.

[0172] TOPOLOGY: Linear.

[0173] MOLECULE TYPE: Synthetic oligonucleotide.

[0174] PROPERTIES: Used for assembly of CDR-grafted heavy chain.

45 TCCAGATCCA GGGTCAATCC ATCCCATCCA CTCGAGTCCC TGTCCAGGTG 50
CCTGTCTCAT CCAATTAATG TAGTAGTCGG TGAAGGTGTA TCCAGA 96

50 SEQUENCE ID NO: 12.

[0175] SEQUENCE TYPE: Nucleotide.

SEQUENCE LENGTH: 93.

55 [0176] STRANDEDNESS: Single.

[0177] TOPOLOGY: Linear.

[0178] MOLECULE TYPE: Synthetic oligonucleotide.

EP 0 781 845 B1

[0179] PROPERTIES: Used for assembly of CDR-grafted heavy chain.

5 GTAGTAGTAG GTGGTCTTCT CTCTTGCACA GAAGTAGAAT GCTGTGTCCT 50
CAGATCTCAG AGAAGACAGC TCCATGTAG CGGTATTCGT GGA 93

SEQUENCE ID NO: 13.

[0180] SEQUENCE TYPE: Nucleotide.

SEQUENCE LENGTH: 21.

[0181] STRANDEDNESS: Single.

[0182] TOPOLOGY: Linear.

[0183] MOLECULE TYPE: Synthetic oligonucleotide.

[0184] PROPERTIES: Used for assembly of CDR-grafted heavy chain.

[0185] FEATURES: Apal site at residues 7 - 12.

20 GCGCGCGGGC CCTTCGTTGA G 21

SEQUENCE ID NO: 14.

[0186] SEQUENCE TYPE: Amino acid.

SEQUENCE LENGTH: 139.

[0187] MOLECULE TYPE: Immunoglobulin heavy chain variable domain.

[0188] FEATURES: CDRs at residues 45 - 54, 69 - 85 and 118 - 128

Kabat numbering }
35 CDR2
10 Pro Gly Ser Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe 48
30 Thr Asp Tyr Tyr Ile Asn Trp Met Arg Gln Ala Pro Gly Gln Gly Leu 64
46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76
Glu Trp Met Gly Trp Ile Asp Pro Gly Ser Gly Asn Thr Lys Tyr Asn 80
77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129
Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ser Thr Asn 96
Tyr Phe Cys Ala Arg Glu Lys Thr Thr Tyr Tyr Tyr Ala Met Asp Tyr 112
Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser 139
CDR1
CDR2
CDR3

SEQUENCE ID NO: 15.

[0189] SEQUENCE TYPE: Nucleotide.

SEQUENCE LENGTH: 21.

[0190] STRANDEDNESS: Single.

[0191] TOPOLOGY: Linear.

[0192] MOLECULE TYPE: Synthetic oligonucleotide.

[0193] PROPERTIES: Used for assembly of CDR-grafted light chain.

[0194] FEATURES: BstBI site at residues 7 to 11

GGACTGTTTCG AAGCCGCCAC C

21

5 SEQUENCE ID NO: 16.

[0195] SEQUENCE TYPE: Nucleotide.

SEQUENCE LENGTH: 81.

10

[0196] STRANDEDNESS: Single.

[0197] TOPOLOGY: Linear.

[0198] MOLECULE TYPE: Synthetic oligonucleotide.

[0199] PROPERTIES: Used for assembly of CDR-grafted light chain.

15

TGGCTTACAG ATGCCAGATG CGATATCCAG ATGACTCAGA GTCCAAGTAC
TCTCAGTCCC AGTGTAGGTG ATAGGGTCAC C

50

81

20

SEQUENCE ID NO: 17.

[0200] SEQUENCE TYPE: Nucleotide.

25

SEQUENCE LENGTH: 90.

[0201] STRANDEDNESS: Single.

[0202] TOPOLOGY: Linear.

[0203] MOLECULE TYPE: Synthetic oligonucleotide.

30

[0204] PROPERTIES: Used for assembly of CDR-grafted light chain.

GGTGACACCT TCCTCTATTG GTTCCAGCAG AAACCAGGTA AAGCCCCAAA
GCTCCTCATG TATAGGATGA GTAACCTCGC CAGTGGTGTA

50

90

35

SEQUENCE ID NO: 18.

[0205] SEQUENCE TYPE: Nucleotide.

40

SEQUENCE LENGTH: 99.

[0206] STRANDEDNESS: Single.

[0207] TOPOLOGY: Linear.

45

[0208] MOLECULE TYPE: Synthetic oligonucleotide.

[0209] PROPERTIES: Used for assembly of CDR-grafted light chain

CAGCCAGATG ATTTCCGCCAC TTATTATTGT ATGCAGCATC TCGAATATCC
ATCACTTTC GGTCAGGGTA CTAAAGTAGA AGTAAAACGT ACGGGCCGG

50

99

50

SEQUENCE ID NO: 19.

55

[0210] SEQUENCE TYPE: Nucleotide.

EP 0 781 845 B1

SEQUENCE LENGTH: 81.

[0211] STRANDEDNESS: Single.
 [0212] TOPOLOGY: Linear.
 5 [0213] MOLECULE TYPE: Synthetic oligonucleotide.
 [0214] PROPERTIES: Used for assembly of CDR-grafted light chain.
 [0215] FEATURES: BstBI site at residues 70 to 75.

10 GCATCTGGCA TCTGTAAGCC ACAGCAGCAG GAGTCCGAGG ACTTGGGTGG 50
 GGACAGACAT GGTGGCGGCT TCGAACAGTC C 81

SEQUENCE ID NO: 20.

15 [0216] SEQUENCE TYPE: Nucleotide.

SEQUENCE LENGTH: 81.

20 [0217] STRANDEDNESS: Single.
 [0218] TOPOLOGY: Linear.
 [0219] MOLECULE TYPE: Synthetic oligonucleotide.
 [0220] PROPERTIES: Used for assembly of CDR-grafted light chain.

25 CCAATAGAGG AAGGTGTCAC CGTTACTATG GAGGAGACTT TTACTACTCC 50
 TACAAGTGAT GGTGACCCTA TCACCTACAC T 81

SEQUENCE ID NO: 21.

30 [0221] SEQUENCE TYPE: Nucleotide.

SEQUENCE LENGTH: 102.

35 [0222] STRANDEDNESS: Single.
 [0223] TOPOLOGY: Linear.
 [0224] MOLECULE TYPE: Synthetic oligonucleotide.
 [0225] PROPERTIES: Used for assembly of CDR-grafted light chain.

40 AGTGGCGAAA TCATCTGGCT GGAGACTACT GATACTGAGA GTGAACTCAG 50
 TACCACTACC ACTACCACTG AATCTAGATG GTACACCACT GGCGAGGTTA 100
 CT 102

45 SEQUENCE ID NO: 22.

[0226] SEQUENCE TYPE: Nucleotide.

50 SEQUENCE LENGTH: 21.

[0227] STRANDEDNESS: Single.
 [0228] TOPOLOGY: Linear.
 [0229] MOLECULE TYPE: Synthetic oligonucleotide.
 55 [0230] PROPERTIES: Used for assembly of CDR-grafted light chain.
 [0231] FEATURES: SphI site at residues 7 - 12.

CCGGCCCCGTA CGTTTACTT C

21

5 SEQUENCE ID NO: 23.

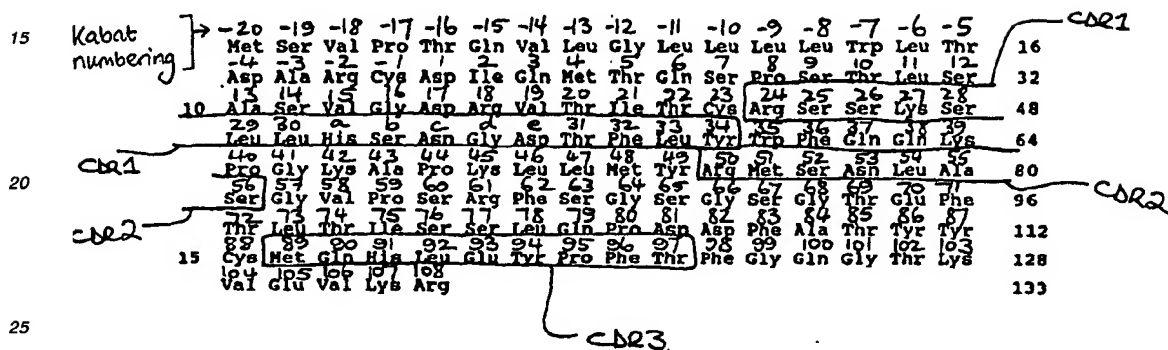
[0232] SEQUENCE TYPE: Aminoacid.

SEQUENCE LENGTH: 133.

10

[0233] MOLECULE TYPE: Immunoglobulin light chain variable domain.

[0234] FEATURES: CDRs at residues 44 - 59, 75 - 81 and 114 - 122



30 Claims

1. A DNA sequence which encodes the composite heavy chain of an antibody molecule having specificity for human milk fat globule (HMFG), wherein:

35 the variable domain of said composite heavy chain comprises predominantly framework region residues from a human immunoglobulin or an analogue thereof;
amino acid residues 2, 26 to 35, 37, 50 to 65, 71, 73, 95 to 105 and 107 (according to the Kabat numbering system) at least in said heavy chain variable domain are derived from the corresponding residues in the monoclonal antibody CTMO1 (as shown in SEQ ID NO: 1); and
40 the remaining immunoglobulin derived parts of the heavy chain are derived from a human immunoglobulin or an analogue thereof.

2. A DNA sequence which encodes the composite heavy chain of an antibody molecule having specificity for human milk fat globule (HMFG), wherein:

45 the variable domain of said composite heavy chain comprises predominantly framework region residues from a human immunoglobulin or an analogue thereof;
amino acid residues 2, 26 to 35, 37, 48, 50 to 65, 67, 69, 71, 73, 95 to 105 and 107 (according to the Kabat numbering system) at least in said heavy chain variable domain are derived from the corresponding residues in the monoclonal antibody CTMO1 (as shown in SEQ ID NO: 1); and
50 the remaining immunoglobulin derived parts of the heavy chain are derived from a human immunoglobulin or an analogue thereof.

3. The DNA sequence of claim 1 or claim 2, wherein additionally in said composite heavy chain at least one of residues 6, 23, 49, 76, 78, 80, 88 and 91 are derived from the corresponding residues in the monoclonal antibody CTMO1 (as shown in SEQ ID NO: 1).

4. A DNA sequence which encodes the composite light chain of an antibody molecule having specificity for human

milk fat globule (HMFG), wherein:

the variable domain of said composite light chain comprises predominantly framework region residues from a human immunoglobulin or an analogue thereof;
 amino acid residues 3, 24 to 34, 36, 50 to 56, 63, 91 to 96 and 108 (according to the Kabat numbering system) at least in said light chain variable domain are derived from the corresponding residues in the monoclonal antibody CTMO1 (as shown in SEQ ID NO: 2); and
 the remaining immunoglobulin derived parts of the light chain are derived from a human immunoglobulin or an analogue thereof.

5. A DNA sequence which encodes the composite light chain of an antibody molecule having specificity for human milk fat globule (HMFG), wherein:

the variable domain of said composite light chain comprises predominantly framework region residues from a human immunoglobulin or an analogue thereof;
 amino acid residues 3, 24 to 34, 36, 37, 45, 48, 50 to 56, 63, 91 to 96 and 108 (according to the Kabat numbering system) at least in said light chain variable domain are derived from the corresponding residues in the monoclonal antibody CTMO1 (as shown in SEQ ID NO: 2); and
 the remaining immunoglobulin derived parts of the light chain are derived from a human immunoglobulin or an analogue thereof.

6. The DNA sequence of claim 4 or claim 5, wherein additionally in said composite light chain, residues 89, 90 and 97 are derived from the corresponding residues in the monoclonal antibody CTMO1 (as shown in SEQ ID NO: 2).

7. The DNA sequence of any one of claims 4 to 6, wherein additionally in said composite light chain, at least one of the residues 1, 2, 49, 60, 70, 84, 85 and 87 are derived from the corresponding residues in the monoclonal antibody CTMO1 (as shown in SEQ ID NO: 2).

8. The DNA sequence of any one of claims 1 to 7, wherein the human framework residues in the composite light chain variable domain or composite heavy chain variable domain are derived from the human LAY, POM, TUR, TEI, KOL, NEWM, REI or EU variable domain sequences.

9. The DNA sequence of any one of claims 1 to 3 or claim 8, wherein the constant region of the heavy chain is of human IgG class.

10. The DNA sequence of claim 9, wherein the constant region of the heavy chain is of human IgG4 subclass.

11. The DNA sequence of claim 9, wherein the constant region of the heavy chain is of human IgG4 subclass with a proline residue at position 241.

12. The DNA sequence of any one of claims 4 to 8, wherein the constant domain of the light chain is of the human kappa class.

13. The DNA sequence of any one of claims 1 to 3 or 8 to 11 which encodes the heavy chain of a Fab, Fab', F(ab')₂, or Fv fragment.

14. The DNA sequence of any one of claims 4 to 8 or 12 which encodes the light chain of a Fab, Fab', F(ab')₂, or Fv fragment.

15. The DNA sequence of any one of claims 1 to 12 which encodes a single chain Fv fragment.

16. A cloning or expression vector containing DNA sequence(s) according to any one of claims 1 to 15.

17. A host cell transformed with (i) DNA sequence(s) according to any one of claims 1 to 15 or (ii) a cloning or expression vector according to claim 16.

Patentanspruch

1. DNA-Sequenz, welche die zusammengesetzte schwere Kette eines Antikörpermoleküls mit Spezifität für menschliche Milchfettkügelchen (HMFG) kodiert, wobei

5 die variable Domäne der zusammengesetzten schweren Kette vorallem Gerüstregionreste von einem menschlichen Immunglobulin oder einem Analogon davon umfaßt,

die Aminosäurereste 2, 26 bis 35, 37, 50 bis 65, 71, 73, 95 bis 105 und 107 (nach dem Kabat-Numerierungssystem) mindestens in der variablen Domäne der schweren Kette von den korrespondierenden Resten im monoklonalen Antikörper CTM01 (wie in SEQ ID Nr. 1 gezeigt) abgeleitet sind und

10 die restlichen Immunglobulin-abgeleiteten Teile der schweren Kette von einem menschlichen Immunglobulin oder einem Analogon davon abgeleitet sind.
2. DNA-Sequenz, welche die zusammengesetzte schwere Kette eines Antikörpermoleküls mit Spezifität für menschliche Milchfettkügelchen (HMFG) kodiert, wobei

15 die variable Domäne der zusammengesetzten schweren Kette vorallem Gerüstregionreste von einem menschlichen Immunglobulin oder einem Analogon davon umfaßt,

die Aminosäurereste 2, 26 bis 35, 37, 48, 50 bis 65, 67, 69, 71, 73, 95 bis 105 und 107 (nach dem Kabat-Numerierungssystem) mindestens in der variablen Domäne der schweren Kette von den korrespondierenden Resten im monoklonalen Antikörper CTM01 (wie in SEQ ID Nr. 1 gezeigt) abgeleitet sind und

20 die restlichen Immunglobulin-abgeleiteten Teile der schweren Kette von einem menschlichen Immunglobulin oder einem Analogon davon abgeleitet sind.
3. DNA-Sequenz nach Anspruch 1 oder Anspruch 2, wobei zusätzlich in der zusammengesetzten schweren Kette mindestens einer der Reste 6, 23, 49, 76, 78, 80, 88 und 91 von den korrespondierenden Resten im monoklonalen Antikörper CTM01 (wie in SEQ ID Nr. 1 gezeigt) abgeleitet ist.

25
4. DNA-Sequenz, welche die zusammengesetzte leichte Kette eines Antikörpermoleküls mit Spezifität für menschliche Milchfettkügelchen (HMFG) kodiert, wobei

30 die variable Domäne der zusammengesetzten leichten Kette vorallem Gerüstregionreste von einem menschlichen Immunglobulin oder einem Analogon davon umfaßt,

die Aminosäurereste 3, 24 bis 34, 36, 50 bis 56, 63, 91 bis 96 und 108 (nach dem Kabat-Numerierungssystem) mindestens in der variablen Domäne der leichten Kette von den korrespondierenden Resten im monoklonalen Antikörper CTM01 (wie in SEQ ID Nr. 2 gezeigt) abgeleitet sind und

35 die restlichen Immunglobulin-abgeleiteten Teile der leichten Kette von einem menschlichen Immunglobulin oder einem Analogon davon abgeleitet sind.
5. DNA-Sequenz, welche die zusammengesetzte leichte Kette eines Antikörpermoleküls mit Spezifität für menschliche Milchfettkügelchen (HMFG) kodiert, wobei

40 die variable Domäne der zusammengesetzten leichten Kette vorallem Gerüstregionreste von einem menschlichen Immunglobulin oder einem Analogon davon umfaßt,

die Aminosäurereste 3, 24 bis 34, 36, 37, 45, 48, 50 bis 56, 63, 91 bis 96 und 108 (nach dem Kabat-Numerierungssystem) mindestens in der variablen Domäne der leichten Kette von den korrespondierenden Resten im monoklonalen Antikörper CTM01 (wie in SEQ ID Nr. 2 gezeigt) abgeleitet sind und

45 die restlichen Immunglobulin-abgeleiteten Teile der leichten Kette von einem menschlichen Immunglobulin oder einem Analogon davon abgeleitet sind.
6. DNA-Sequenz nach Anspruch 4 oder Anspruch 5, wobei zusätzlich in der zusammengesetzten leichten Kette die Reste 89, 90 und 97 von den korrespondierenden Resten im monoklonalen Antikörper CTM01 (wie in SEQ ID Nr. 2 gezeigt) abgeleitet sind.

50
7. DNA-Sequenz nach einem der Ansprüche 4 bis 6, wobei zusätzlich in der zusammengesetzten leichten Kette mindestens einer der Reste 1, 2, 49, 60, 70, 84, 85 und 87 von den korrespondierenden Resten im monoklonalen Antikörper CTM01 (wie in SEQ ID Nr. 2 gezeigt) abgeleitet ist.
- 55 8. DNA-Sequenz nach einem der Ansprüche 1 bis 7, wobei die menschlichen Gerüstreste in der variablen Domäne der zusammengesetzten leichten Kette oder der variablen Domäne der zusammengesetzten schweren Kette von den variablen Domänen der menschlichen Sequenzen LAY, POM, TUR, TEI, KOL, NEWM, REI oder EU abgeleitet sind.

9. DNA-Sequenz nach einem der Ansprüche 1 bis 3 oder Anspruch 8, wobei die konstante Region der schweren Kette aus der menschlichen IgG-Klasse ist.
- 5 10. DNA-Sequenz nach Anspruch 9, wobei die konstante Region der schweren Kette aus der menschlichen IgG4-Unterklasse ist.
11. DNA-Sequenz nach Anspruch 9, wobei die konstante Region der schweren Kette aus der menschlichen IgG4-Unterklasse mit einem Prolinrest in Position 241 ist.
- 10 12. DNA-Sequenz nach einem der Ansprüche 4 bis 8, wobei die konstante Domäne der leichten Kette aus der menschlichen Kappa-Klasse ist.
13. DNA-Sequenz nach einem der Ansprüche 1 bis 3 oder 8 bis 11, welche die schwere Kette eines Fab-, Fab'-, F(ab')₂- oder Fv-Fragments kodiert.
- 15 14. DNA-Sequenz nach einem der Ansprüche 4 bis 8 oder 12, welche die leichte Kette eines Fab-, Fab'-, F(ab')₂- oder Fv-Fragments kodiert.
15. DNA-Sequenz nach einem der Ansprüche 1 bis 12, welche ein Einzelketten-Fv-Fragment kodiert.
- 20 16. Klonierungs- oder Expressionsvektor, welcher die DNA-Sequenz(en) nach einem der Ansprüche 1 bis 15 enthält.
17. Wirtszelle, die mit (i) DNA-Sequenz(en) nach einem der Ansprüche 1 bis 15 oder (ii) einem Klonierungs- oder Expressionsvektor nach Anspruch 16 transformiert ist.
- 25

Revendications

- 30 1. Séquence d'ADN qui code pour la chaîne lourde composite d'une molécule d'anticorps ayant une spécificité pour le globule de matières grasses de lait humain (HMFG), dans laquelle:

le domaine variable de ladite chaîne lourde composite comprend principalement des résidus de la région charpente d'une immunoglobuline humaine ou d'une de ses analogues;
35 au moins les résidus d'acides aminés 2, 26 à 35, 37, 50 à 65, 71, 73, 95 à 105 et 107 (selon le système de numérotation de Kabat) dudit domaine variable de chaîne lourde sont dérivés des résidus correspondants dans l'anticorps monoclonal CTMO1 (comme indiqué dans SEQ ID NO: 1); et
les parties restantes dérivées d'immunoglobuline de la chaîne lourde sont dérivées d'une immunoglobuline humaine ou d'une de ses analogues.
- 40 2. Séquence d'ADN qui code pour la chaîne lourde composite d'une molécule d'anticorps ayant une spécificité pour le globule de matières grasses de lait humain (HMFG), dans laquelle:

le domaine variable de ladite chaîne lourde composite comprend principalement des résidus de la région charpente d'une immunoglobuline humaine ou d'une de ses analogues;
45 au moins les résidus d'acides aminés 2, 26 à 35, 37, 48, 50 à 65, 67, 69, 71, 73, 95 à 105 et 107 (selon le système de numérotation de Kabat) dudit domaine variable de chaîne lourde sont dérivés des résidus correspondants dans l'anticorps monoclonal CTMO1 (comme indiqué dans SEQ ID NO: 1); et
les parties restantes dérivées d'immunoglobuline de la chaîne lourde sont dérivées d'une immunoglobuline humaine ou d'une de ses analogues.
- 50 3. Séquence d'ADN selon la revendication 1 ou la revendication 2, dans laquelle en outre dans ladite chaîne lourde composite, au moins l'un des résidus 6, 23, 49, 76, 78, 80, 88 et 91 est dérivé des résidus correspondants dans l'anticorps monoclonal CTMO1 (comme indiqué dans SEQ ID NO: 1).
- 55 4. Séquence d'ADN qui code pour la chaîne légère composite d'une molécule d'anticorps ayant une spécificité pour le globule de matières grasses de lait humain (HMFG), dans laquelle:

le domaine variable de ladite chaîne légère composite comprend principalement des résidus de la région

charpente d'une immunoglobuline humaine ou d'une de ses analogues;
au moins les résidus d'acides aminés 3, 24 à 34, 36, 50 à 56, 63, 91 à 96 et 108 (selon le système de numérotation de Kabat) dudit domaine variable de chaîne légère sont dérivés des résidus correspondants dans l'anticorps monoclonal CTMO1 (comme indiqué dans SEQ ID NO: 2); et
les parties restantes dérivées d'immunoglobuline de la chaîne légère sont dérivées d'une immunoglobuline humaine ou d'une de ses analogues.

5. Séquence d'ADN qui code pour la chaîne légère composite d'une molécule d'anticorps ayant une spécificité pour le globule de matières grasses de lait humain (HMFG), dans laquelle:

le domaine variable de ladite chaîne légère composite comprend principalement des résidus de la région charpente d'une immunoglobuline humaine ou d'une de ses analogues;
au moins les résidus d'acides aminés 3, 24 à 34, 36, 37, 45, 48, 50 à 56, 63, 91 à 96 et 108 (selon le système de numérotation de Kabat) dudit domaine variable de chaîne légère sont dérivés des résidus correspondants dans l'anticorps monoclonal CTMO1 (comme indiqué dans SEQ ID NO: 2); et
les parties restantes dérivées d'immunoglobuline de la chaîne légère sont dérivées d'une immunoglobuline humaine ou d'une de ses analogues.

6. Séquence d'ADN selon la revendication 4 ou la revendication 5, dans laquelle en outre dans ladite chaîne légère composite, les résidus 89, 90 et 97 sont dérivés des résidus correspondants dans l'anticorps monoclonal CTMO1 (comme indiqué dans SEQ ID NO: 2).

7. Séquence d'ADN selon l'une quelconque des revendications 4 à 6, dans laquelle en outre dans ladite chaîne légère composite, au moins un des résidus 1, 2, 49, 60, 70, 84, 85 et 87 est dérivé des résidus correspondants dans l'anticorps monoclonal CTMO1 (comme indiqué dans SEQ ID NO: 2).

8. Séquence d'ADN selon l'une quelconque des revendications 1 à 7, dans laquelle les résidus de la région charpente humaine dans le domaine variable de chaîne légère composite ou le domaine variable de chaîne lourde composite sont dérivés des séquences à domaines variables LAY, POM, TUR, TEI, KOL, NEWM, REI ou EU humains.

9. Séquence d'ADN selon l'une quelconque des revendications 1 à 3 ou la revendication 8, dans laquelle la région constante de la chaîne lourde appartient à la classe des IgG humaines.

10. Séquence d'ADN selon la revendication 9, dans laquelle la région constante de la chaîne lourde appartient à la sous-classe des IgG4 humaine.

11. Séquence d'ADN selon la revendication 9, dans laquelle la région constante de la chaîne lourde appartient à la sous-classe des IgG4 humaine avec un résidu proline en position 241.

12. Séquence d'ADN selon l'une quelconque des revendications 4 à 8, dans laquelle le domaine constant de la chaîne légère appartient à la classe kappa humaine.

13. Séquence d'ADN selon l'une quelconque des revendications 1 à 3 ou 8 à 11, qui code pour la chaîne lourde d'un fragment Fab, Fab', F(ab')₂ ou Fv.

14. Séquence d'ADN selon l'une quelconque des revendications 4 à 8 ou 12, qui code pour la chaîne légère d'un fragment Fab, Fab', F(ab')₂ ou Fv.

15. Séquence d'ADN selon l'une quelconque des revendications 1 à 12, qui code pour un fragment Fv à chaîne unique.

16. Vecteur de clonage ou d'expression contenant une ou plusieurs séquences d'ADN selon l'une quelconque des revendications 1 à 15.

17. Cellule hôte transformée avec (i) une ou plusieurs séquences d'ADN selon l'une quelconque des revendications 1 à 15 ou (ii) un vecteur de clonage ou d'expression selon la revendication 16.

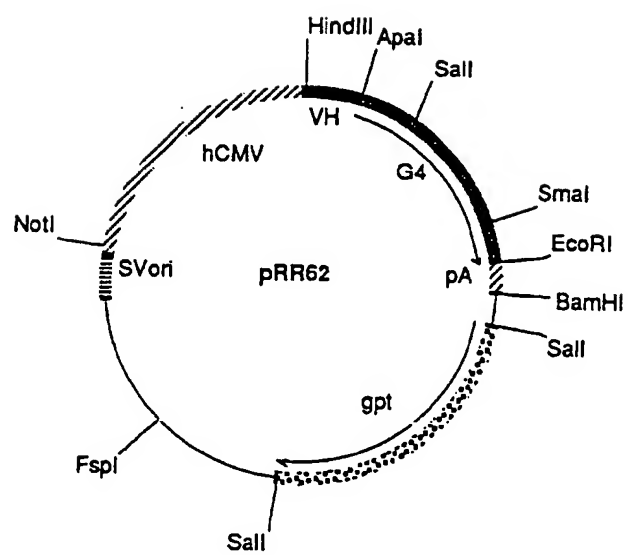


FIG. 1

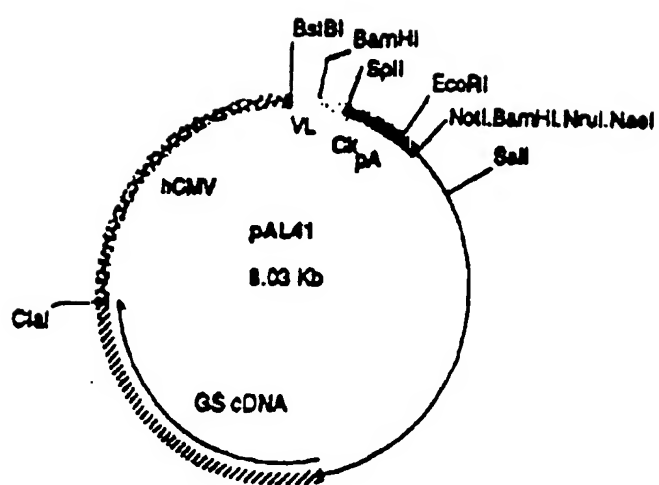


FIG. 2

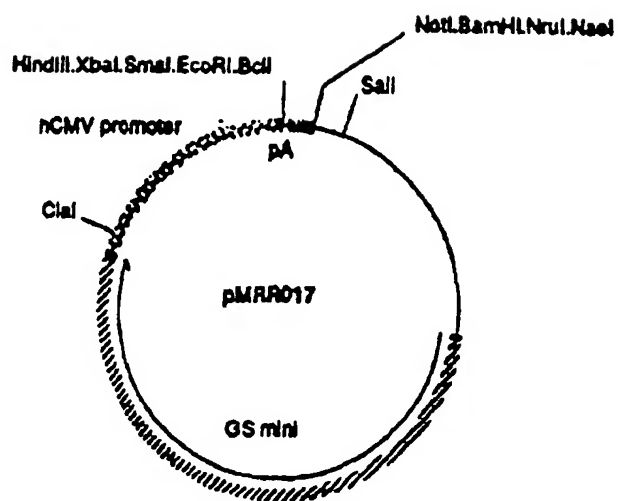


FIG. 3

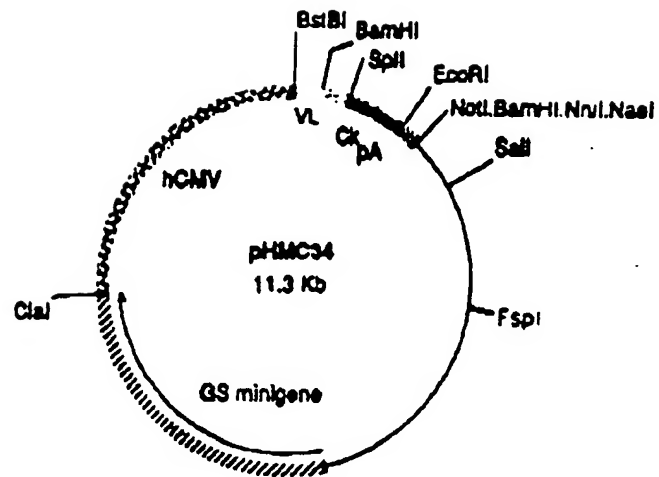


FIG. 4

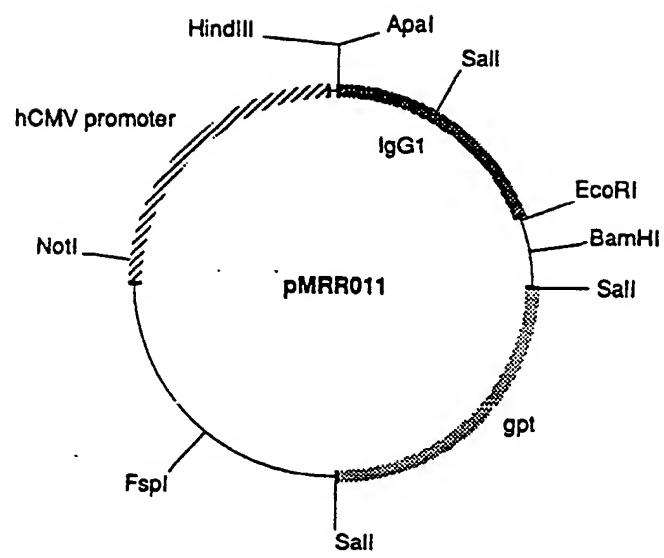


FIG. 5

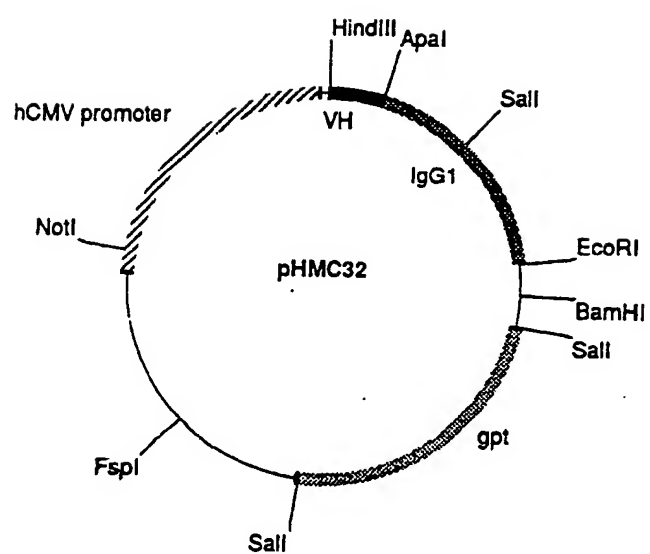


FIG. 6

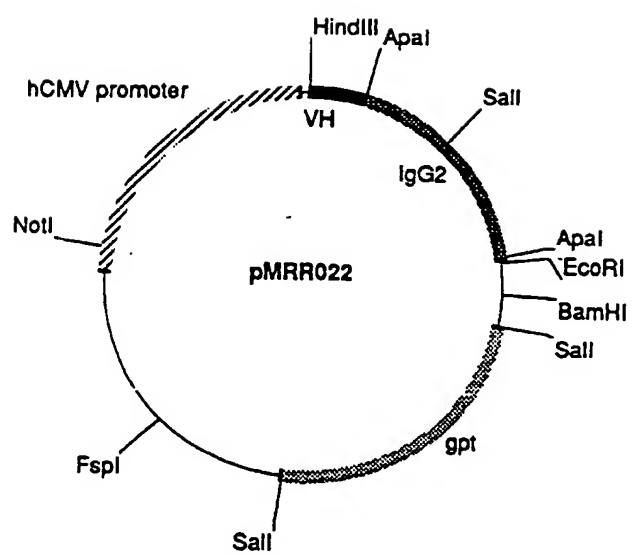


FIG. 7

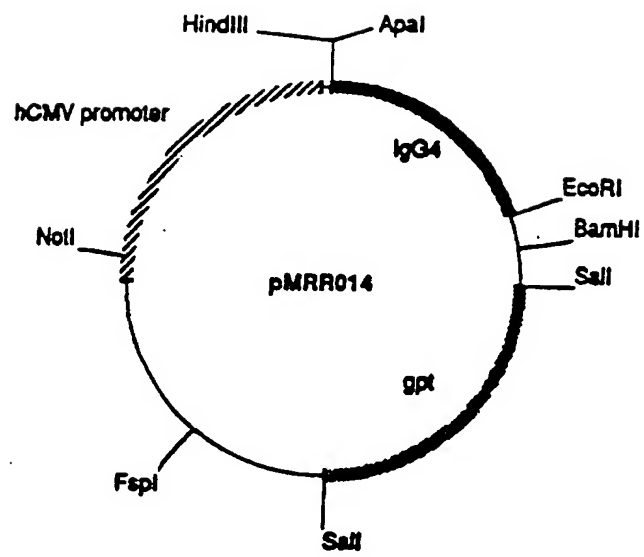


FIG. 8

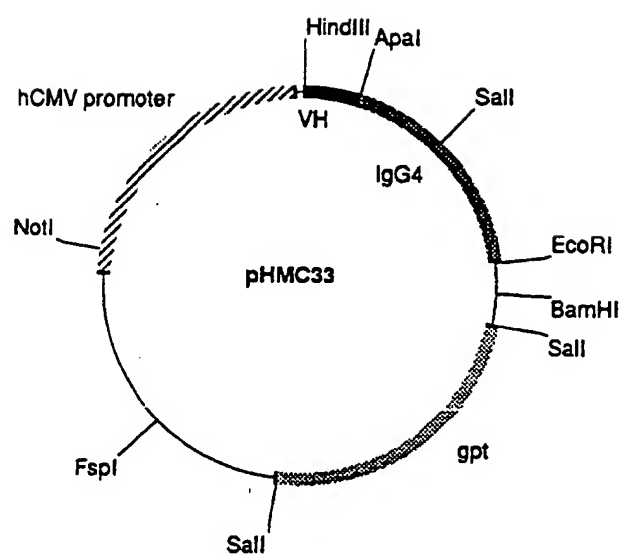


FIG. 9

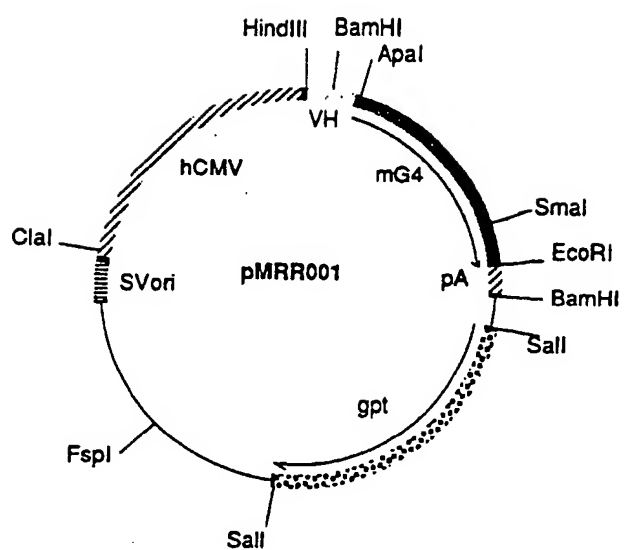


FIG. 10

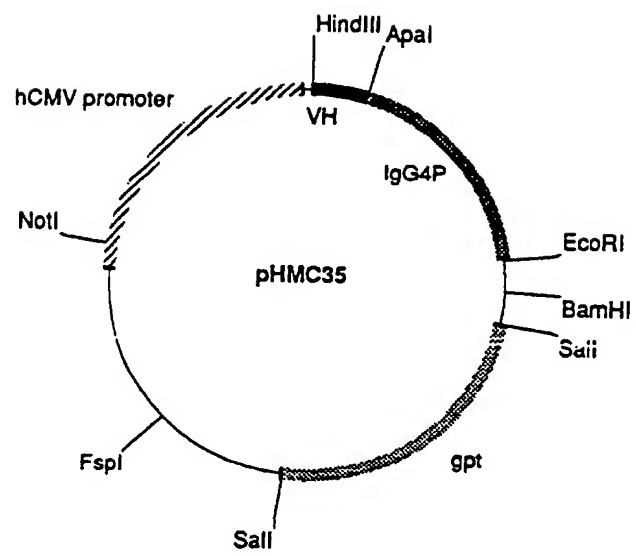


FIG. 11

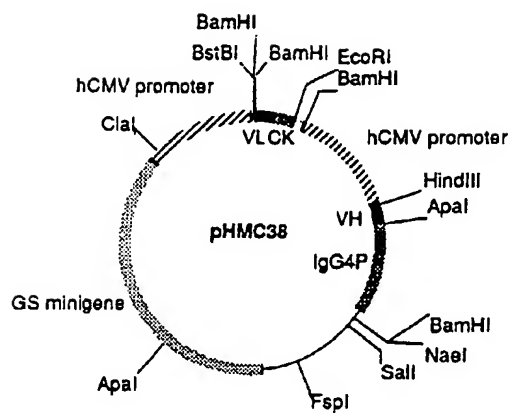


FIG. 12

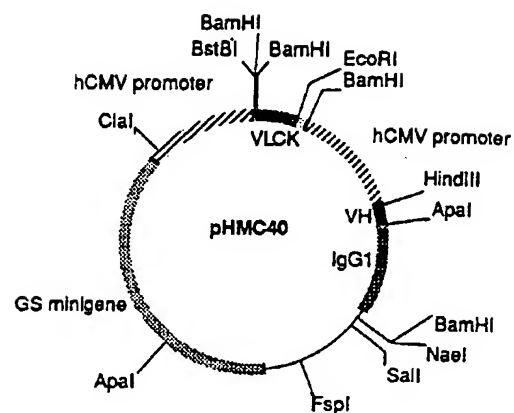


FIG. 13

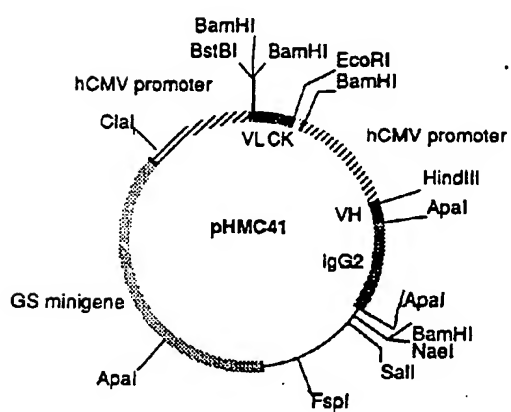


FIG. 14

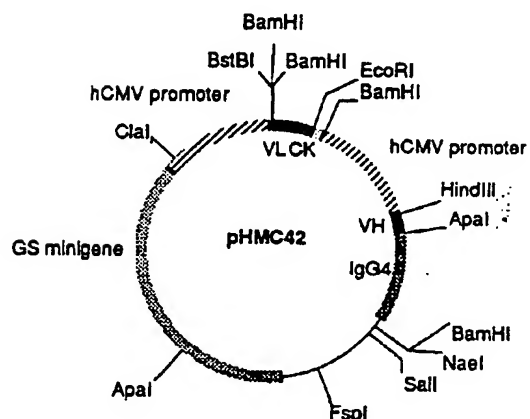


FIG. 15

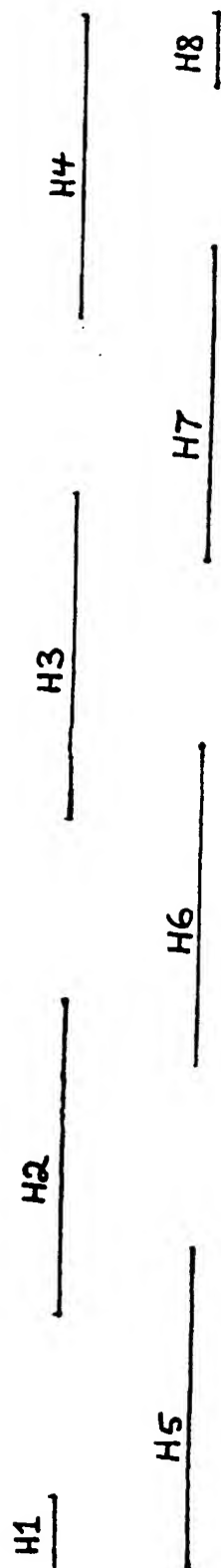


FIG. 16

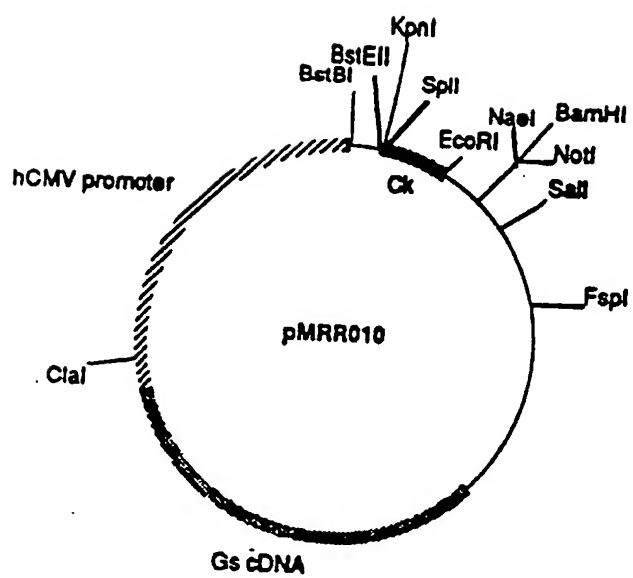


FIG. 19

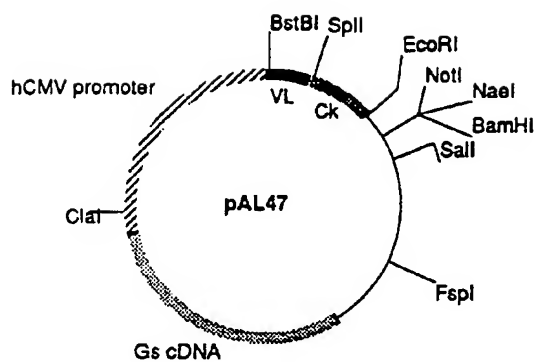


FIG. 20

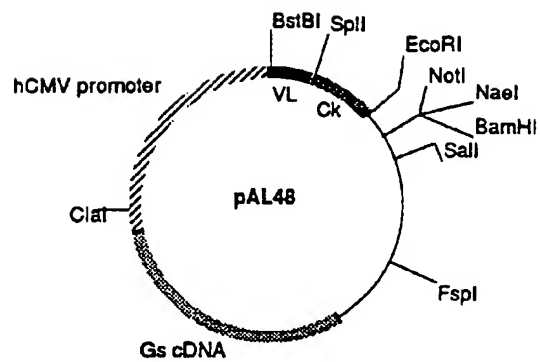


FIG. 21

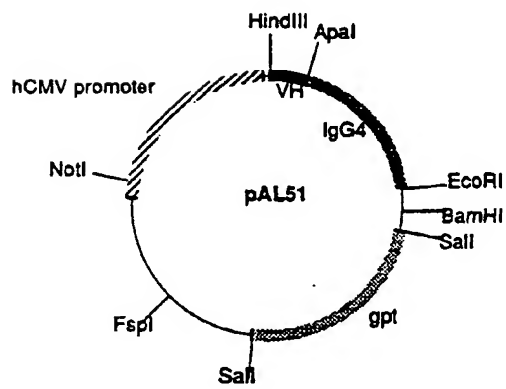


FIG. 17

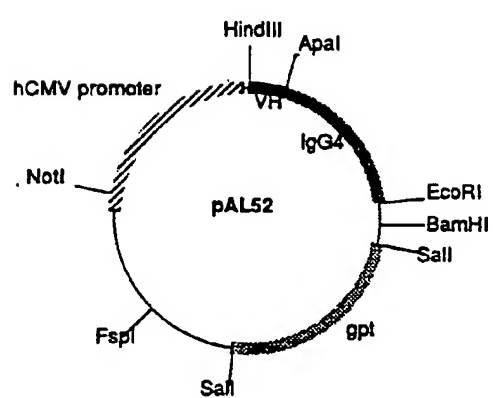
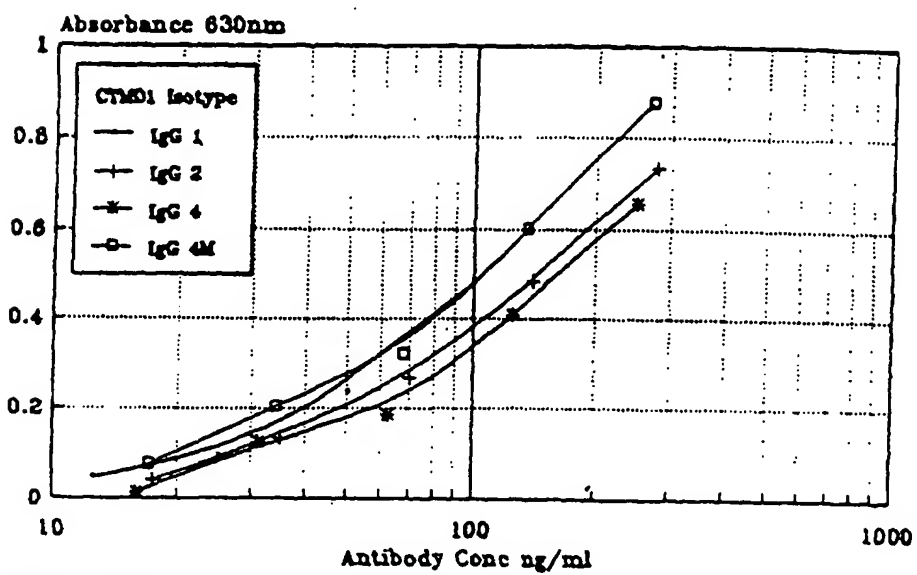


FIG. 18

Direct Binding ELISA: Anti-PEM Activity of Chimeric CTM01 Subclass Series



A. LAWSON 4.2.91

FIG. 22

Direct Binding ELISA: Anti-milk PEM
Activity of Humanized CTM01

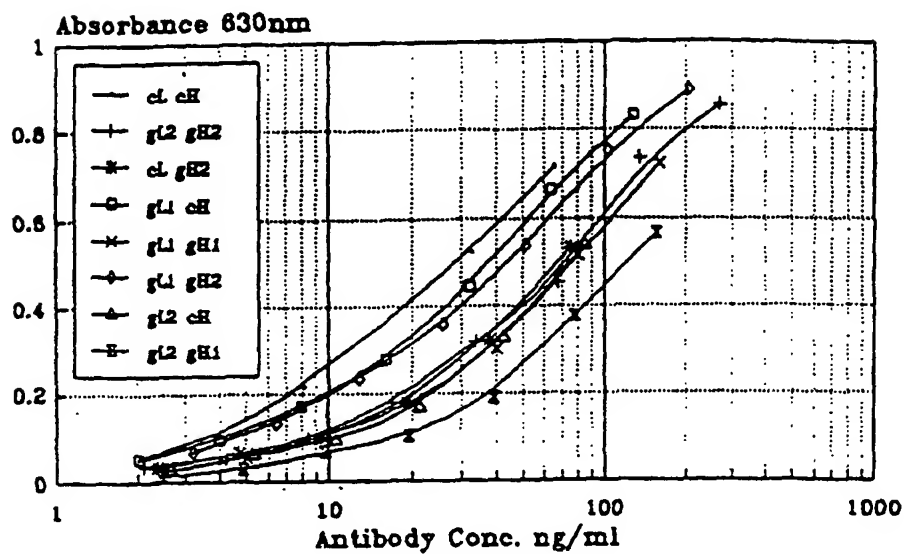


FIG. 23

Anti-PEM Competition EIA
Biotin-murine CTM01 vs PEM solid phase

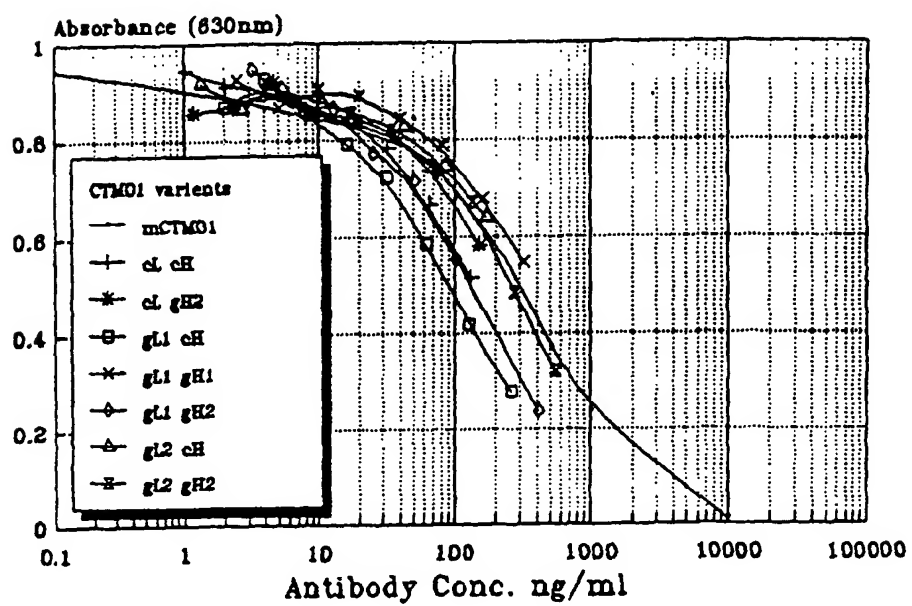


FIG. 24